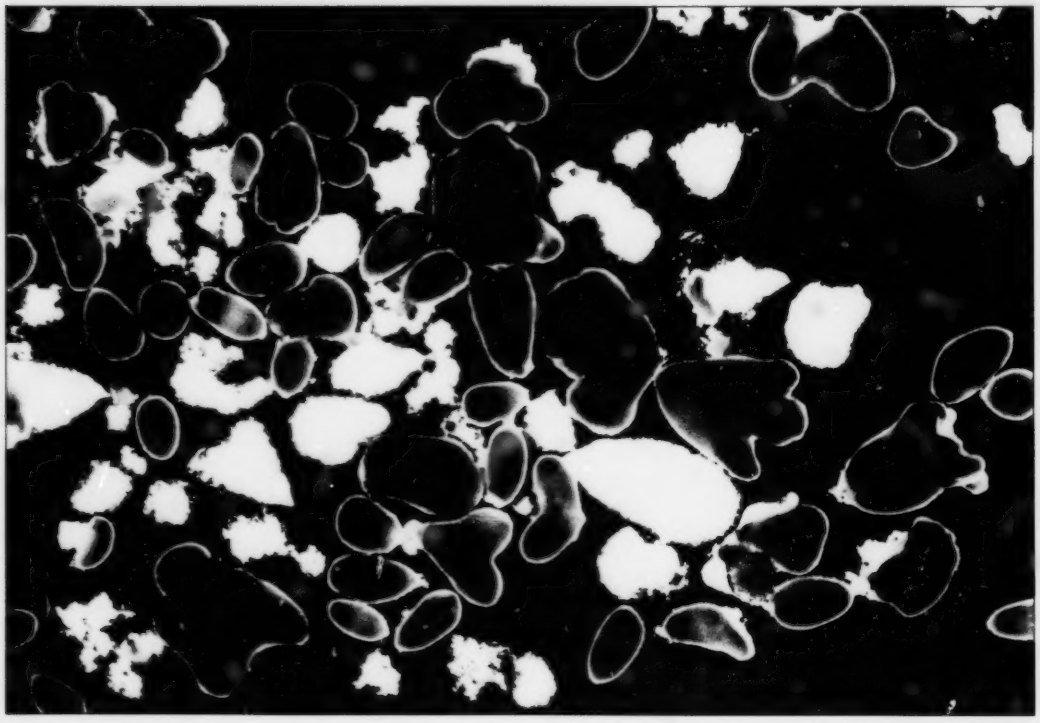
National Research Council Canada
Conseil national de recherches Canada

Plant Biotechnology Institute

Annual Report 1989-1990



Canada

91-04028/

The National Research Council in Biotechnology

The National Research Council of Canada (NRC) is a federal crown-owned corporation created in 1916 to promote, assist and perform research of economic and social benefit to the nation. Today NRC operates more than a dozen national laboratories across Canada, along with programs for scientific-technical information and industrial research assistance.

Since 1924 the Council has maintained a strong program in biosciences, and in 1983 was designated by the Canadian government to lead the national effort in biotechnology research. To this end the programs of the Biological Sciences Institute (Ottawa), the Biotechnology Research Institute (Montreal), the Plant Biotechnology Institute (Saskatoon) and the Marine Sciences Institute (Halifax) have been brought together in a unified Biotechnology Program - this last laboratory joining the Program in 1989.

In this program the Plant Biotechnology Institute has a mission to deliver new, exploitable biological and biochemical methods and materials for the control and transgenic alteration of plant development, through research especially at the cellular and molecular levels.

This mission reflects NRC's purpose under the National Research Council Act, to "promote research (i)...on the utilization of the natural resources of Canada; (ii) with the object of...discovering processes and methods that may promote the expansion of existing or the development of new industries; (iii) ...to improve conditions in agriculture."

National Research Council of Canada
Plant Biotechnology Institute
Annual Report 1989/90

Contents

1. Highlights of the year 1989/90 2
2. Organization and personnel 4
   - 2.1 Organization
   - 2.2 The Board of Governors
   - 2.3 Staff, Affiliates and Guests
3. Cell Technology Section 8
   - 3.1 Overview
   - 3.2 Crop biotechnology project
   - 3.3 Conifer biotechnology project
4. Gene Technology Section 16
   - 4.1 Overview
   - 4.2 Stress tolerance genes project
   - 4.3 Regulation of gene expression in plant project
   - 4.4 Legume biotechnology project
5. Biological Chemistry Section 24
   - 5.1 Overview
   - 5.2 Bio-organic chemistry project
   - 5.3 Seed oil modification project
   - 5.4 Plant cell metabolism project
6. Transgenic Plant Centre 33
   - 6.1 Overview
   - 6.2 Personnel and facilities
7. Business and Outreach Section 34
   - 7.1 Overview
   - 7.2 Technology development
   - 7.3 Outreach activities
8. Support Services 38
   - 8.1 Overview
   - 8.2 Service units
   - 8.3 Committees
9. Publications and Patents in 1989 40
10. Canada's Investment at PBI, 1989/90 44

1. Highlights of the year 1989/90

The Plant Biotechnology Institute (PBI) of the National Research Council of Canada (NRC) is one of four NRC centres for biotechnology research and development. During this seventh year of operation we made very substantial progress in our core research program, and in assistance to other Canadian biotechnology R&D groups. This report describes our main activities and achievements through the year spanning April 1989 to March 1990.

Our Institute fills two needs on the Canadian biotechnology scene. On one hand it brings together sizeable teams -- typically 12 to 20 people -- to work on specific projects important to Canada. Usually these teams are directly linked with industrial research partners. On the other hand, PBI provides outstanding research facilities in which many organizations have now sited research personnel, or even whole projects. In these two ways the Institute contributes to that "national competitiveness" which has become an issue in Canada.

Plant genetic engineering is the main focus of the Institute, by which we are providing the agriculture and forestry sectors with novel plants containing heritable new traits. Technologies and methods needed for the use of such new plants are another concern of our Institute program.

Success in these areas requires research activity on four fronts. First, a mastery of plant cell culture techniques is vital. Second, effective means for introducing new genes (or gene-controlling DNA sequences) into cells are required for the genetic transformation of species. Third, the ability to regenerate artificially cultured cells into complete plants is needed in order to recover "transgenic" plants from transformation experiments. Fourth, ways to control and regulate the action of genes resident in plants -- whether they are present naturally or have been introduced through transformation -- are of the utmost importance in creating novel plant prototypes which will perform satisfactorily in the field or forest. These four methodologies of cell culture, transformation, plant regeneration and gene regulation underlay most of our research in 1989/90.

We continue to develop methods of particular applicability to four important classes of Canadian plants : cereal grains, legume grains, *Brassica* oilseeds and conifer trees. The experimentation described in this report therefore deals mostly with these plant types, which have proven to be the main interest areas also of our industry partners.

PBI kept its staff size of 110 despite financial constraints at NRC, and during 89/90 acted as host to nearly one hundred guest researchers from universities and industries. These guests were associated in our project efforts and contributed a great deal to the success of these efforts. Indeed, the maintenance of our guests by their organizations represented an investment of $2.6 million during the year. It has been extremely gratifying that so many Canadian organizations have chosen PBI as a site for their plant biotechnology research operations.

Our laboratories produced a wealth of new scientific and technical advances during the year, reported in nearly one hundred publications. These discoveries included :

* The first ever transformation of barley cells was achieved, and methods for regeneration of cereal grain plants were improved (see Chapter 3);

* In studies of salt tolerance, genes encoding trimethylglycine biosynthesis were isolated and their gene product structures deduced; trimethylglycine plays a role in salt tolerance (see Chapter 4);

* DNA regulatory elements were discovered which allow tissue-specific and developmental-specific expression of genes in plants. Applications of these elements in agronomically useful systems has begun (see Chapter 4);

* In research on embryogenesis of conifer trees from cultured cells, sample spruce has been produced in sufficient quantities for nursery tests, and methods for embryogenesis were improved (see Chapter 3);

* Procedures were worked out for transformation of pea and for regeneration of pea plants, and these methods were extended to other grain legume species (see Chapter 4);

* Experiments with abscisic acid and its metabolites have thrown much light on how abscisic acid exerts its various effects in plants under stress (see Chapter 5);

* In seed oil modification research, microspore embryos were discovered to provide an excellent model system for study of storage lipid biosynthesis. Also the capability of Brassica species to form trierucin from 1,2-dierucin was demonstrated (see Chapter 5);

* A plant gene encoding tryptophan decarboxylase was defined and used in genetic engineering this function (see Chapter 5).

The Institute took a decisive step forward in 1989/90 by establishing a large new facility for plant propagation and assessment. This "Transgenic Plant Centre" comprises greenhouses designed specifically for handling transgenic research material, and associated laboratories for on-site experimentation with them. More than half of the facility has been earmarked for use in industrial collaborations and by PBI's plant biotechnology partners. Our existing facilities for research were also extended and improved by renovating several older areas of the Institute to make laboratories for collaborative research projects. Rapid growth of PBI's industrial partnerships was evident in the year's revenue and contract values which rose to more than double those of 1988/89.

University collaborations also grew. By year-end more than half of all PBI Research Officers had been awarded adjunct professorships at one or another Canadian university. Conversely, eleven outstanding university researchers had become Affiliates of the Institute, and a record level of student thesis research was being carried out in our laboratories. By the close of the year, the Institute was operating at a high level of productivity which probably cannot be increased much further without more core staff.

Research success depends ultimately upon the excellence of researchers. Our PBI staff scientists received many honours during the year, some of which are listed in Chapter 6. In particular, Drs Kuo-Nan Kao and Sean Hemmingsen stood at the top of the most-cited Canadian plant biotechnology researchers in 1989. While Drs Vincenzo DeLuca and Robert Tyler left PBI to take up university appointments in Canada, we were fortunate in having Drs Wilfred Keller and David Taylor join our staff in the latter part of the year.

At the opening of the 1990s, NRC's Plant Biotechnology Institute continues to be a catalyst for cross-sectoral collaborations and for industrial developments in plant biotech throughout Canada.

Warren Steck
Director General

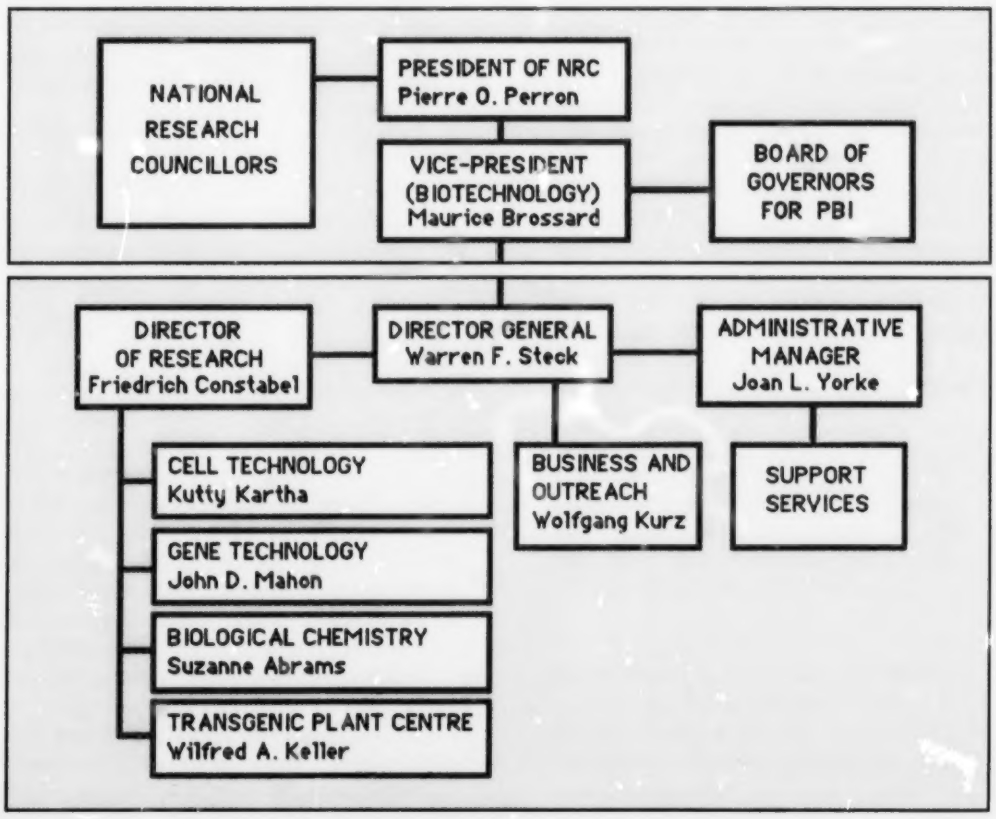
2. Organization and Personnel

2.1 Organization

Our Institute's organization is shown in the diagrams below and on the next page. PBI has four laboratory sections each specialized for particular research methods, and two smaller business-related sections. The figure below shows these sections and current heads. Each section is the base for project groups of which nine existed in 1989/90. These are shown in the chart on page 5.

The Board of Governors represents various players in the Canadian research community with interests in plant biotechnology; the Board's function is to advise NRC's Vice-President (Biotechnology) on issues relating to the Institute. The Board met only once in 1989/90.

PBI's core staff numbered 110 through the year, of whom 86 were laboratory personnel. These 86 included twenty-seven permanent Ph.D. scientists and thirty-eight Technical Officers, along with twenty term-appointed Ph.D. scientists and five other limited-term positions. Twelve summer students were present in 1989. Our non-NRC guests numbered 85 over the year. (Explanations of the nature of Guest Researchers and Affiliated Scientists appear in Chapter 7.)



2.2 The Board of Governors

Dr. Guy BELLEMARE, *Professor, Faculty of Science, Laval University (Sainte-Foy, Quebec)*
Dr. Wallace BEVERSDORF, *Professor and Chairman, Department of Crop Science, University of Guelph (Guelph, Ontario)*
Dr. Maurice BROSSARD, *Vice President Science /Biotechnology (NRC), Chairman of the Board of Governors.*
Dr. Ian DE LA ROCHE, *Assistant Deputy Minister, Western Economic Diversification Canada (Saskatoon, Saskatchewan)*
Prof. Leo KRISTJANSON, *Past President, University of Saskatchewan (Saskatoon, Saskatchewan)*
Dr. Maurice LALONDE, *Professor of Biology, Laval University (Sainte-Foy, Quebec)*
Dr. Kelvin OGILVIE, *Vice President (Academic) Acadia University (Wolfville, Nova Scotia)*
Dr. Warren STECK, *Director General, NRC Plant Biotechnology Institute*
Dr. Ted TURNER, *Chancellor, University of Saskatchewan (Saskatoon, Saskatchewan).*
Mr. Gordon WELLS, *President, Tradex International (Calgary, Alberta).*
Dr. John WILLIAMS, *Professor of Botany, University of Toronto (Toronto, Ontario)*

2.3 Staff, Affiliates and Guests

2.3.1 Staff of the Institute 1989/90

An asterisk (*) indicates a limited-term staff appointment. Some staff were not present for the entire year.

DIRECTOR GENERAL: *Warren F. STECK, B.Eng. (McGill); Ph.D. (Saskatchewan)*

SECRETARY TO THE DIRECTOR GENERAL: *Carol WALKER*

RESEARCH DIRECTOR: *Friedrich CONSTABEL, Dr. Res. Nat. (Gottingen), Dr. Habil. (Bonn)*

ADMINISTRATIVE MANAGER: *Joan L. YORKE*

PRINCIPAL RESEARCH OFFICERS:
Wolfgang G.W. KURZ, Ph.D. (Vienna)
Edward W. UNDERHILL, B.S.P., M.Sc. (Sask.) Ph.D. (Rhode Island)

SENIOR RESEARCH OFFICERS:
William L. CROSBY, B.Sc. (British Columbia), Ph.D. (Edinburgh)
David I. DUNSTAN, B.Sc. (London), Ph.D. (NE London Polytechnic)
Kuo-Nan KAO, B.S. (Chung-Hsing, China), M.S.A. (Guelph), Ph.D. (Saskatchewan)
Kutty K. KARTHA, B.Sc. (Saugar), M.Sc. (Jawaharlal), Ph.D. (New Delhi)
Wilfred A. KELLER, B.S.A., Ph.D. (Saskatchewan)
Samuel L. MacKENZIE, B.Sc., Ph.D. (Edinburgh)
John D. MAHON, B.Sc.(Western Ontario), M.Sc.,Ph.D. (Queen's)
George P. SLATER, B.Sc. (Aberdeen), M.Sc. (Saskatchewan), Ph.D. (Queen's, Belfast)

ASSOCIATE RESEARCH OFFICERS:
Suzanne R. ABRAMS, B.Sc. (Carleton), Ph.D. (Dalhousie)
John J. BALSEVICH, B.Sc., M.Sc., Ph.D. (British Columbia)
Ravindra N. CHIBBAR, B.Sc., M.Sc. (Meerrut), Ph.D. (Panjab), Ph.D. (Western Ontario)
Adrian J. CUTLER, B.Sc. (Wales), Ph.D. (Florida State)
Vincenzo DELUCA, B.Sc. (Concordia), M.Sc. (Queen's), Ph.D. (Western Ontario)
Fawzy GEORGES, M.Sc. (Brock), Ph.D. (Ottawa)
Jan W.D. GROOTWASSINK, B.Sc., M.Sc. (Wageningen), Ph.D. (Calgary)
Sean M. HEMMINGSEN, B.Sc. (British Columbia), Ph.D. (Queen's)
Shaun L.A. HOBBS, M.A. (Cambridge), B.A., D. Phil. (Oxford)
Martin S. LAPP, B.Sc., M.Sc. (York), Ph.D. (Alberta)
Mstaslaw MAZUREK, B.S.A. (Saskatchewan)
Louise M. NELSON, B.Sc. (Western Ontario), Ph.D. (Calgary)
Lawrence E. PELCHER, B.S. (Aquinas), M.S., Ph.D. (Mich. State)
Mohammed SALEEM, B.Sc., M.Sc. (Pakistan), M.Sc. (Laurentian), Ph.D. (Western Ontario)
Gopalan SELVARAJ, B.Sc. (Bangalore); M.Sc., Ph.D. (Carleton)
Janet L. TAYLOR, B. Sc. (Iowa State), Ph.D. (Washington State)
Robert T. TYLER, B.S.A., Ph.D. (Saskatchewan)

Research sections of the NRC Plant Biotechnology Institute, 1990

CELL TECHNOLOGY SECTION	GENE TECHNOLOGY SECTION	BIOLOGICAL CHEMISTRY SECTION	TRANSGENIC PLANT CENTRE
Crop biotechnology (K. Kartha)	Stress tolerance genes (G. Selvaraj)	Bio-organic chemistry (S. R. Abrams)	[Propagation and assessment]
Conifer biotechnology (D. I. Dunstan)	Regulation of gene expression in plants (S. M. Hemmingsen)	Seed oil modification (S. L. MacKenzie)	Brassica biotechnology (W. A. Keller) [Starts in 1990]
	Legume biotechnology (J. D. Mahon)	Plant cell metabolism (W. G. W. Kurz)	

ASSISTANT RESEARCH OFFICERS:
David C. TAYLOR, B.Sc., Ph.D. (Carleton)
Edward W.T. TSANG, B.Sc., M.Sc., Ph.D. (Saskatchewan)

RESEARCH COUNCIL OFFICERS:
J. Sylvan KATZ, B.Sc., M.Sc. (Saskatchewan)*
T. Martin MALLARD

VISITING RESEARCH OFFICER
Craig L. NESSLER, B.S., M.A. (William & Mary), Ph.D. (Indiana)*

RESEARCH ASSOCIATES:
Luc ADAM, B.Sc., Ph.D. (Laval)*
Sylvain ADAM, M.Sc. (Orsay), D.E.A., Ph.D. (Paris)*
Faouzi BEKKAOUI, B.Sc. (Berkane), M.Sc.(Tours), Ph.D. (Paris)*
Joseph G. BOOTHE, B.Sc., Ph.D. (Western Ontario)*
Supa CHAVADEJ, B.Sc., M.Eng. (Chulalongkorn), Dr. Res. Nat. (Heidelberg)*
Lynn CLONEY, B.Sc., Ph.D. (New Brunswick)*
Raju S. DATLA, B.Sc., M.Sc., Ph.D. (Andhra)*
Allen GOOD, B.Sc. (Trent), M.Sc.(Windsor), Ph.D. (Ottawa)*
Atta HUSSAIN, B.Sc. (Iraq), M.Sc. (Birmingham), Ph.D. (Aston)*
Jennifer JACKSON, B.Sc. (St. Andrews), Ph.D. (Wales)*
Jinesh C. JAIN, B.Sc. , M.Sc. (Rajasthan), Ph.D. (Jodhpur)*
Mark C. JORDAN, B.Sc. (Guelph), M.Sc. (Manitoba), Ph.D. (Sask.)*
Edward J. KENDALL, B.Sc., M.Sc. (Dalhousie), Ph.D. (Guelph)*
*Lesh KLIMCZAK *,M. Sc.(Biology), Ph.D. (Wurzburg)*
Ljerka KUNST, B.Sc., M.Sc. (Zagreb), Ph.D. (Michigan)*
Nancy LAMB, B.Sc. (Hong Kong), Ph.D. (British Columbia)*
Monika LULSDORF, B. Eng. (Bonn), M.Sc. (Man.), Ph.D. (B.C.)*
Remi MARTEL, B.Sc., M.Sc., Ph.D. (Laval)*
Brenda McAFEE, B.Sc., M.Sc., (Acadia), Ph.D. (Laval)*
Victor MORALES, B.Sc. (Columbia), M.Sc., Ph.D. (Florida)*
Lloyd A.K. NELSON, B.Sc. (Ghana), Ph.D. (Alberta)*
W. Jay NEWSTED, B.Sc., M.Sc., Ph.D. (Western Ontario)*
Nancy PAIVA, B.S. (Harvey Mudd), Ph.D. (M.I.T.)*
M.Soledade C. PEDRAS, B.Sc., M.Sc. (Porto), Ph.D. (Alberta)*
Gregory PENNER, B.S.A., M.Sc.,(Manitoba),Ph.D. (Saskatchewan)*
Gilles PILATE, B. Ed., M.Sc., Ph.D. (Paris)*
Javed QURESHI, B.Sc., M.Sc. (Karachi), Ph.D. (Liverpool)*
Hans REMPEL, B.Sc. (California State), Ph.D. (California)*
David D. SONGSTAD, B.Sc., M.Sc.(South Dakota), Ph.D. (Tenn.)*
Lawrence W. THOMSON, B.Sc., M.Sc., Ph.D. (Alberta)*
Randall S. WESELAKE, B.Sc., M.Sc.,Ph.D. (Manitoba)*

LABORATORY TECHNICAL OFFICERS
Maureen ANDERSON
David BALISKI, B.S.T. (K.I.A.A.S.)
Carla BARBER, B.Sc. (Saskatchewan)
Dennis BARTON, B.Sc. (Saskatchewan)*
Brian BECK, B.Sc. (Carleton)*
Colleen BENNETT, B.S.T. (K.I.A.A.S.)*
Terry BETHUNE, B.Sc. (Saskatchewan)
Greg BISHOP, B.Sc. (Saskatchewan)
Cheryl BOCK, B.Sc. (Saskatchewan)
Ira BORGMANN, B.Sc., M.Sc., (British Columbia)
Karen CASWELL, B.S.A., M.Sc. (Saskatchewan)
K. Brock CHATSON , C.R.T. (S.A.I.T.)
Maxine COFFEY, B.L.T. (Regina)
Janet CONDIE, B.Sc. (Queen's)
Catherine DELONG, B.S.A., B.Sc. (Saskatchewan)
*John DYCK**
Don DYMOND
Delwin EPP B.S.T. (K.I.A.A.S.)*
Joanna FREELAND, B.Sc. (Saskatchewan)*
Laurie FRIESEN B.Sc., M.Sc. (Saskatchewan)*
Mike GIBLIN
Joseph HAMMERLINDL
Rozina HIRJI, B.Sc. (Makerere)
Lawrence HOGGE
Les HOFFMAN
David HORN
Connie KNAPP
Diana KNEESHAW, B.S.A. (Saskatchewan)
Allen KOLENOVSKY, B.Sc. (Saskatchewan)
Nick LEUNG, B.Sc. (Saskatchewan)
Mary LOEWEN, B.S.A. (Saskatchewan)
Lily MacDONALD B.Sc. (Saskatchewan)*
Cliff MALLARD
John MAGUS, B.Sc.(Saskatchewan)
Jana MALINEK, B.Sc., M.Sc. (Palacky)
Morris MICHAYLUK
Debra NOVAKOVSKI, B. Sc. (Saskatchewan)*
Doug OLSON, B.S.T. (K.I.A.A.S.)
Keith PAHL, B.S.T. (K.I.A.A.S.)
Barry PANCHUK
Bob PAPISH, B.Sc. (Saskatchewan)
Sandra POLVI, B.S.T. (K.I.A.A.S.)
Ruth POWER , B. Sc. (Brandon), M. Sc. (Saskatchewan)*
Darwin REED, B.Sc. (Saskatchewan)
Don SCHWAB, B.S.T. (K.I.A.A.S.)
Angela SHAW, B.Sc. (Saskatchewan)
Jerry SHYLUK
Lee STEINHAUER, B.A. (Saskatchewan)
Don TENASCHUK, B.Sc. (Saskatchewan)
Maureen WOOD, B.S.T. (K.I.A.A.S.)*

ADMINISTRATIVE SERVICES
Gayle BERRY
Rosemarie GALLAYS
Dawn LARSON
Debbie McLEOD
Kathryn SEABROOK
Katherine WILSON

SUPPLY SERVICES
Cheryl BITZ
Olga COATES
*Jill HORN**
Irene HOWE
Terry MAZURKEVICH
Jim McHARG
Bill WILEY

RESEARCH ILLUSTRATION:
Ed KNAPP

GENERAL SERVICES:
Bill BRUMWELL
Armand FENDELET
Harry HOLFELD
Rick McDONALD
George REYNOLDS
Wayne STANKEWICH

PUBLICATIONS AND EVENTS:
Lorraine BLASHILL

SUMMER STUDENTS 1989
Nathalie BEAUDOIN, Laval University
Ken CARRUSCA, University of British Columbia
Paul CERMAK, University of Guelph
Graham CLEGHORN, University of Saskatchewan
Normand CLOUTIER, McGill University
Pamela DAVID, University of British Columbia
John DE CARLE, Mount Allison University
Dion DURNFORD, Dalhousie University
Susan LIU, McGill University
Ruth POWER, University of Saskatchewan
Kevin RIOUX, University of Saskatchewan
George SAMUEL , McGill University

2.3.2 Affiliated Scientists of the Institute 1989/90

Helmut BERTRAND, *B.A., M.Sc., Ph.D. (Kansas) Professor of Microbiology, University of Guelph*
Normand BRISSON, *B.Sc., M.Sc. (Montreal), Ph.D. (McGill) Professor of Biochemistry, University of Montreal*
Keith DOWNEY, *B.Sc., M.Sc., (Sask), Ph.D. (Cornell) Head, Oilseeds Section, Agriculture Canada Research Station (Saskatoon, Sask.)*
Lawrence FOWKE, *B.A. (Hons) (Sask), Ph.D. (Carleton) Professor of Biology, University of Saskatchewan*
Bryan L. HARVEY, *B.S.A., M.Sc., (Sask.), PhD (California) Professor of Crop Science & Plant Ecology, University of Saskatchewan; Director of Saskatchewan Crop Development Center*
George HAUGHN, *B.Sc. (Dalhousie), Ph.D. (Cornell) Professor of Biology, University of Saskatchewan*
Brian MIKI, *B.Sc.(Carleton), Ph.D. (Toronto) Plant Research Centre, Agriculture Canada*
Maurice MOLONEY, *B.Sc.(London), Ph.D. (Lesicester) Professor of Biology, University of Calgary*
Ann OAKS, *B.A., M.A. (Toronto), Ph.D. (Saskatchewan) Professor of Biology, McMaster University*
Trevor L. THORPE, *B.Sc. (Allahabad), B.Sc., Ph.D. (California) Professor of Biology, University of Calgary*
G. H. Neil TOWERS, *B.Sc., M.Sc. (McGill), Ph.D. (Cornell) Professor of Biology, University of British Columbia*

2.3.3 PBI Guest Researchers in 1989/90

Dr. Garth Abrams, Crop Development Centre
Dr. Jesus Alvarez, Government of Spain
Ms. Alison Baillie, University of Saskatchewan
Dr. K. Balasubramanian, University of Saskatchewan
Dr. Vipan K. Bansal, Agriculture Canada
Dr. Sant Bhojwani, University of Delhi (India)
Ms. Lesley A. Boyd, University of Saskatchewan
Mr. Robin Browne, University of Saskatchewan
Mr. H. Cheng, Genetic Institute of Jiangsu Academy of Agricultural Sciences, Nanjing (China)
Ms Diana Chenier, University of Saskatchewan
Dr. Normand Chevrier, Université du Québec (Montréal)
Ms. Cheryl Craig, University of Saskatchewan
Mr. Jim Cunningham, Philom Bios Inc.
Dr. Laurence Davin, Université de Paris (France)
Dr. Peter S. S. Dawson, Biotechnology Research Institute
Ms Monique Dethier, Universite catholique de Louvain (Belgium)
Mr John Dyck, professional services
Ms Patricia Englund, Vipont Research Laboratories Inc.
Mr. Delwin Epp, Vipont Pharmaceuticals Inc.
Ms. Laurie Friesen, Vipont Research Laboratories Inc.
Dr. Basu V. Gantotti, Philom Bios Inc.
Dr. Kenneth Giles, Vipont Research Laboratories Inc.
Dr. Larry Gusta, Crop Development Centre
Ms. Cheryl Hampson, University of Saskatchewan
Mr Ronald Harkrader, Vipont Research Laboratories Inc.
Dr. George Haughn, University of Saskatchewan
Mr Dwayne Hegedus, University of Saskatchewan
Dr. Harriet Hemmingsen, Oxford University (U.K.)
Ms. Carol Hill, Vipont Research Laboratories Inc.
Dr. Sjoichi Ichihashi, Aichi University (Japan)
Mr. Ravinder Jain, University of Saskatchewan
Mr. Blair Jarvis, University of Saskatchewan
Ms. Grace Johnson, Saskatchewan Wheat Pool
Mr. Raman Kapur, University of Saskatchewan
Ms. Susan Kikcio, University of Calgary
Dr. John King, University of Saskatchewan
Dr Lesh Klimczak, NSERC
Ms. Kristina Komendant, Philom Bios Inc.
Ms. Cathy Kuiack, Philom Bios Inc.
Dr. Ljerka Kunst, University of Saskatchewan
Dr Nancy Lamb, Crop Development Centre
Ms. Debbie Lashta, Philom Bios Inc.
Ms. Kathy Leier, University of Saskatchewan
Ms. Monika MacHutchon, University of Saskatchewan
Ms. Sourindra N. Maiti, University of Saskatchewan
Dr. Chai Mak, University of Singapore
Mr. Braden Manns, University of Saskatchewan
Ms. M. Luisa Marin, C.I.A.T., Colombia
Ms. Marilyn Martin, University of Saskatchewan
Ms. Linda McGregor, Saskatchewan Wheat Pool
Ms Maria Moore, University of Calgary
Mr. N. Nehra, University of Saskatchewan
Dr. Lloyd A.K. Nelson, NSERC
Mr. Y. Niu, University of Saskatchewan
Ms. Debra Novakovski, Vipont Research Laboratories Inc.
Ms. Laurel O'Connor, Philom Bios Inc.
Dr. Brian Orshinsky, Saskatchewan Wheat Pool
Dr. Jong Park, Vipont Research Laboratories Inc.
Dr Kenneth Pivnick, Agriculture Canada
Ms.Ruth Power, University of Saskatchewan
Mr. Martin Reaney, University of Saskatchewan
Mr. Kevin Rozwadowski, University of Saskatchewan
Ms Norma Ruecker, University of Saskatchewan
Ms Susan Scutchings, University of Saskatchewan
Ms. Debbie Sommer, University of Nebraska
Mr. David Songstad, Vipont Research Laboratories Inc.
Ms. Sharon Sprout, University of Saskatchewan
Mr. Jim Sutherland, professional services
Mr. Stephen Tallevi, Bioméga Ltée
Mr. Tom Tautorus, Stange Canada Inc.
Mr. Mike Thompson, University of Calgary
Dr. Leena Toivonen, Finnish Academy of Science
Dr. H.-C. Wang, University of Saskatchewan
Mr. Hong Wang, University of Saskatchewan
Mr. Roger Warren, University of Saskatchewan
Mr. Michael Whiting, University of Saskatchewan
Dr. David Williams, University of Saskatchewan
Ms. Linda Wong, University of Saskatchewan
Mr. David Woodske, Philom Bios Inc.
Ms. Adrienne Woytowich, University of Saskatchewan
Mr. Guohai Wu, University of Saskatchewan
Ms. Melinda Yiu, University of Saskatchewan
Mr. Lu Zhang, University of Saskatchewan

Abbreviations

Biotechnology abounds with abbreviations, and some of these appear in this Report. We give the full term at its first appearance, but the following chart of some frequently-met abbreviations may be helpful:

ABA Abscisic acid
ALS Acetolactate synthase (enzyme)
CoA Coenzyme A
cDNA Complementary deoxyribonucleic acid
GUS Beta-glucuronidase (enzyme)
HPLC High-performance liquid chromatography
HPT hygromycin phosphotransferase (enzyme)
NPT neomycin phosphotransferase (enzyme)
mRNA Messenger ribonucleic acid

3. Cell Technology Section

Section Head: K. K. Kartha

3.1 Overview

The Cell Technology Section grew out of the Institute's pioneering work on *in vitro* plant cell cultures during the 1960s and 1970s. Now, after a quarter century, the Section's activities are largely focussed on methods for transformation and regeneration of plant cells from coniferous and monocotyledonous grain species. These types have long been recognized as difficult but important targets for biotechnology. The facilities of the Section permit *in vitro* culture manipulations, greenhouse propagation, growth chamber experimentation, research in cytology and cell biology, along with genetic engineering capabilities. Core NRC work with crop plants goes forward in the cereal grains biotechnology project and the project on cold tolerance in plants; forest trees are the subject of activities in conifer biotechnology. The first two of these projects are closely interlinked and are reported here under the heading Crop Biotechnology.

The Section now has eleven laboratory units, all with first-class facilities for cell and molecular biology research. During 1989/90 these facilities were further improved by acquisition of an improved micro-ballistic particle gun and of additional controlled environment chambers for experimentation. In addition to its core complement of 31 NRC staff, the Section was host this year to more than twenty extramural guests, and to two industry research teams.

3.2 Crop Biotechnology Project

3.2.1 Personnel

Research Officers: *K.K. Kartha (Leader), A. Cutler, R. Chibbar, F. Georges, K.N. Kao, M. Saleem (on leave)*
Research Associates: *J. Boothe, E. Kendall, J. Newsted, J. Qureshi*
Technical Officers: *K. Caswell, M.A. Coffey, D. Horn, N.L. Leung, M.K. Loewen, C.S. Mallard, M. Michay'uk, B. Papish, K. Pahl, S. Polvi, L. Steinhauer*
Guest Researchers: *A. Baillie, S. Bhojwani, K. Friesen, G. Johnson, S. Ichihashi, R. Kapur, C. Lusignan, L. McGregor, K. Malik, N. Nehra, Y. Niu, B. Orshinsky, R. Power, H. Wang, H.C. Wang, G. Wu, J. Yang, J.M. Ye*
Summer Assistants : *P. Cermak, J. DeCarle, R. Power*

3.2.2 Introduction

During the project year 1989/90, the research activities carried out by the project fell under three major categories: plant regeneration, selection and field testing (wheat, barley, sunflower); genetic engineering of cereal crops (wheat, barley); improvement of cold tolerance (wheat, canola).

Our Project was set up in 1984 with the overall goal of genetic improvement of selected Canadian cereal crop species (wheat, barley) through advanced cell culture, cell selection and genetic engineering technologies. The specific objectives are the creation of these cereal crop species with improved agronomic traits such as resistance to cold and herbicides, along with some parallel applications in sunflower and canola. During the project year 1989/90, and as a first in

Haploid barley from anther culture

Canada, we entered into genetic engineering of cereal crops.

3.2.3 Plant Regeneration, Selection and Field Testing (K.N. Kao, K. Kartha)

High frequency plant regeneration from *in vitro* cultured cells and tissues is a prerequisite for most applications of biotechnology to crop species, however major cereals such as wheat and barley are extremely recalcitrant to plant regeneration attempts. Over the past few years, PBI has made excellent progress in obtaining high efficiency plant regeneration from *in vitro* cultured immature embryos of several cultivars of wheat and barley. Culturing *in vitro* allows plant cells to grow in an unorganized manner, giving rise to variability known as somaclonal variation. We assessed the usefulness of this variation as a breeding tool, using seed progeny of tissue culture regenerants of wheat and barley in extensive field evaluations. Overall we concluded that somaclonal variation is not a promising approach for cereals.

During 1989, large scale field trials of barley regenerant lines were conducted in collaboration with the Crop Development Centre, University of Saskatchewan. More than a thousand lines from embryo culture-derived plants were compared with controls. No significant differences were observed between the tissue culture lines and the control lines. This indicates that the embryo culture procedure did not hinder the performance of the regenerant lines or cause an increase in variation for the traits measured. This observation is very significant and encouraging from the point of view of our ongoing genetic engineering research where genetic stability of the recipient cell system is necessary for the introduction and expression of novel genes.

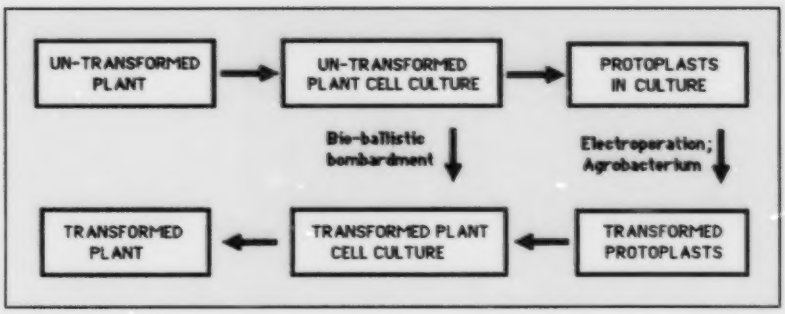
Double haploid plants: Haploid plants containing half the usual number of chromosomes can be produced through *in vitro* culture either of anthers or microspores at appropriate stages of their development. Haploids are extremely valuable material for plant breeders because homozygous pure lines result upon doubling of the chromosomes. Use of haploidy in varietal development programs can reduce the time required to produce new varieties.

Under the terms of an IRAP-assisted project with Saskatchewan Wheat Pool, work continued to develop haploidy systems for various wheats and for use in breeding novel traits. During 1989, several lines of doubled haploids underwent field evaluation in the Pool's breeding program.

Barley Haploidy: In anther culture experiments with barley, higher frequency of plant formation per anther was obtained when maltose, instead of sucrose, was used in media. Attempts to culture barley microspores, instead of whole anthers, induced division of microspores leading to the production of clusters of cells. We stabilized these dividing microspores and made them develop into micro-calli at high frequency. A similarly high frequency of cell division in durum wheat microspores was also observed.

Sunflower: Sunflower experimentation was directed at *in vitro* plant regeneration from immature embryos and with hypocotyl protoplasts. Acceptable regeneration frequency was achieved, and some explant material from plants grown in the Transgenic Plant Centre gave quite exceptional results, all somatic embryos growing green shoots.

Sunflower protoplasts isolated from hypocotyls gave some calli, a few of which produced a root. This protocol is now the most advanced anywhere, but must yet lead to whole plant regeneration in order to be useful. (This work was carried out in collaboration with visiting scientist G.G. Li from the People's Republic of China.)



Genetic engineering by gene insertion requires the intermediacy of plant cell or protoplast cultures, with regeneration of complete plants.

3.2.4 Genetic Engineering of Cereals-Wheat, Barley (R. Chibbar, K. Kartha)

Introduction of foreign genes into plants has been achieved in a variety of plant species. However, cereal grains are not infected by *Agrobacterium tumefaciens* and other methods of transformation fail for lack of efficient regeneration of plants from protoplasts. We explored two strategies to effect transformation in cereals: gamete-mediated transformation, and microprojectile bombardment.

In gamete-mediated transformation, our success in permeabilization of wheat sperm cells through electroporation established the feasibility of introducing foreign genes, and we began optimizing conditions for electroporation. Progress with the isolation of female gametes was more rapid than expected. From embryo sacs mechanically dissected from unfertilized ovules, enzymatic digestion liberated protoplasts among which putative egg cells were identified. This cleaed the way for fertilization experiments using both intact embryo sacs and protoplasts. Research at the University of Alberta (in the laboratory of our collaborator David Cass) is aimed at the development of culture and conditions to maintain viable fertilized embryo sacs/egg cells.

Via microprojectile bombardment, nucleic acids can be delivered directly into intact cells or tissues. We acquired a 'Biolistic Particle Gun' in 1989, and have put a major effort in this approach to cereal biotechnology.

Transient Expression of Marker Genes in Barley and Wheat: The success of direct delivery techniques of nucleic acid to genetically engineer plants requires post-transformation regeneration of a whole plant, so our efforts concentrated on a system with a capacity for high-frequency regeneration: immature zygotic embryos.

Plasmids carrying a wide spectrum of marker genes were assembled for this work on cereal transformation. The main components of constructs used in this work were marker genes (CAT, NPT, GUS or HPT) driven by a constitutive promoter, with an Adh1-intron1 between the promoter and coding region of the marker gene, which is followed by a NOS terminator (see glossary of abbreviations, on page 7).

Transient expression of marker genes was observed in most of the recipient wheat and barley systems; the presence of intron1 of the alcohol dehydrogenase gene considerably enhanced the expression of CAT gene in both systems. The expression of introduced DNA was generally proportional to the amount of DNA introduced into the cells in suspension culture, although such a direct correlation could not be observed with CAT gene in immature zygotic embryos of barley. The nearness of recipient material to the gun stopping plate had some positive effect on the transient expression of CAT gene. On the other hand double firings had deleterious effects.

Detection of CAT expression proved more difficult in wheat than in barley. This very low level of CAT activity was attributed to the presence of an inhibitor of the enzyme in wheat, which could be substantially overcome by heating at 65°C.

The development of high frequency plant regeneration systems in both wheat and barley, coupled with the demonstration of gene expression via direct gene delivery, now paves the way for controlled gene insertion and expression of novel traits in hitherto unresponsive major Canadian crops such as wheat and barley. The year's research at PBI now places the Institute in the forefront of cereal biotechnology in Canada.

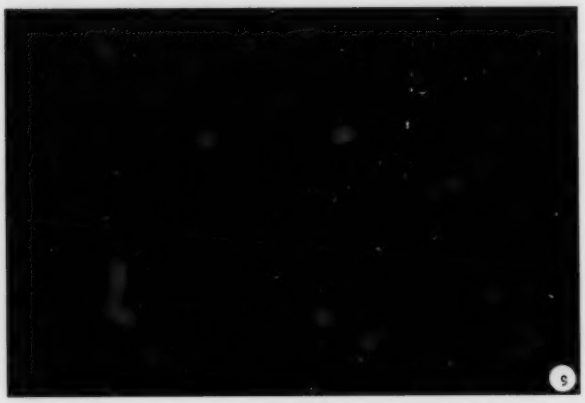
3.2.5 Plant Cold Tolerance (A. Cutler, F. Georges, K. Kartha, M. Saleem)

Improvement of cold tolerance in Canadian crops (wheat, canola) has been a priority area of the project from its inception. In order to accomplish this

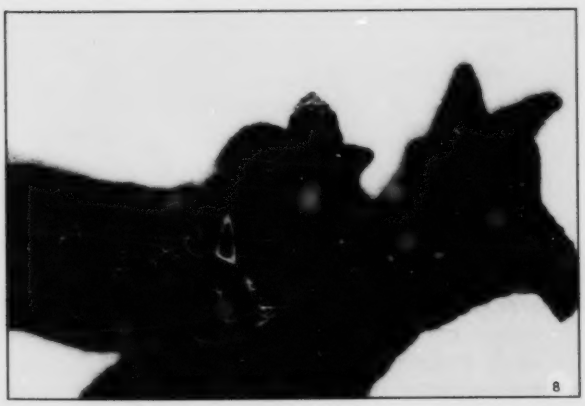
objective, three approaches were pursued during 1989/90: cryoselection (ultra-low temperature selection) of spring wheat; carbohydrate-mediated cold tolerance; and use of antifreeze proteins.

Cryoselection of Spring Wheat: Wheat plants, regenerated from eleven callus lines that had been cryoselected under a range of conditions, were grown to maturity and the seeds collected. We screened seedlings for freezing tolerance (at -12°C.) and found that five of the lines possessed a high level of freezing tolerance (LT50 near -12°C) compared with non-selected controls (LT50 -8°C). In fact, all but two of the test lines showed better survival than control (non-selected) plants. This was the first time selection for freezing tolerance carried out on cultured cells had successfully provided freezing tolerant plants. Furthermore, we found that the trait was transmitted to the seed progeny of these regenerated plants.

Biochemical analysis of one of the cryoselected callus lines revealed the presence of nine polypeptides that were not apparent in similar extracts from non-selected callus. No differences in fatty acid composition were detected between non-selected and cryoselected calli. On the other hand, while both control and selected calli had a similar distribution of monosaccharides in soluble carbohydrate extracts the sucrose concentration was twice as high in cryoselected callus.



The root from transgenic strawberry expressing the GUS gene at the apical region (blue colour).



Stably transformed strawberry.

Carbohydrate-Mediated Cold Tolerance: A monoclonal antibody has been successfully developed against wheat germ sucrose synthetase enzyme. This antibody cross-reacted with sucrose synthetase from several other plant species, including shoots of wheat, maize, barley, pea and from carrot cell culture. The primary purpose for producing the monoclonal antibody against sucrose synthetase was to develop a specific probe for assaying the levels of the synthetase in spring and winter wheat in relation to what is already known about sucrose levels under cold temperature stress. Western blot analysis using the antibody indicated that both winter and spring wheat shoots accumulate synthetase as a function of time spent at 2°C.

We examined the expression of the synthetase at the mRNA level. Northern analysis of total RNA from both wheat varieties, using a synthetic DNA probe homologous with the gene from maize, indicated little or no transcript present in the control shoots. However, a significant level was observed in both varieties after one week at 2°C.

Antifreeze proteins: We earlier had synthesized an effective antifreeze gene based on the sequence for the flounder antifreeze protein (AFP). This gene had been expressed in protoplasts following electroporation. A major problem left over from sequence1988/89 was unambiguous detection of the antifreeze protein-CAT fusion protein in extracts of electroporated maize protoplasts. This was solved by a large scale phenol-based protein extraction. Expression of the fusion protein was detected by western blotting using both anti-CAT and anti-AFP sera. We showed that the AFP-CAT protein was expressed at a similar level to the unfused CAT and found no evidence for enhanced proteolytic sensitivity of the fusion protein. We failed to obtain transient expression in Brassica suspension culture protoplasts even with GUS-based expression vectors, however, results similar to those described in maize were obtained with soybean.

3.2.6 Extramural Collaboration

University collaborations brought many guests to our laboratories. One student, N. Nehra, (University of Saskatchewan) carried out Ph.D. thesis research during the course of which he developed excellent plant regeneration systems using two approaches: a direct shoot regeneration from strawberry leaf discs and regeneration from immature leaf derived callus.

During 1989, these efficient plant regeneration systems were employed to produce transgenic strawberry plants. An efficient genetic transformation protocol using *Agrobacterium tumefaciens* was first developed for the strawberry variety 'Red Coat'. Molecular analysis of each transgenic clone confirmed the integration of NPT and GUS genes into strawberry genome. Leaf explants taken from transgenic strawberry plants formed callus on kanamycin-containing media and this callus also expressed the marker genes. Thus one university collaboration resulted in the first genetic engineering of strawberry.

The Project also continued extensive collaborations with the University of Saskatchewan (Crop Development Centre, Crop Science Department, Department of Horticulture Science, Department of Biology), University of Alberta and the University of Guelph in various aspects of crop biotechnology research. An agreement between PBI and the Crop Development Centre continued in 1989/90, under which the Centre carried out field evaluation and characterization of barley material generated through anther culture and immature embryo culture.

Industrial Collaborations

Our cereal biotechnology activities continued to be carried out in close collaboration with the Saskatchewan Wheat Pool, under the terms of a joint-venture agreement. For this program, the Pool provided expertise and field facilities not available at PBI, including a valuable field testing component.

Additionally, under the terms of an IRAP agreement between Saskatchewan Wheat Pool and NRC, the Pool carried out its biotechnology program on wheat in our laboratories with Dr. Kartha acting as a scientific advisor. This initiative employs three Pool personnel over three years and complements the NRC in-house program.

Saskatchewan's Agriculture Development Fund is funding activities in the Crop Biotechnology Project connected with the production of cold tolerant canola.

3.3 Conifer Biotechnology Project

3.3.1 Personnel:

Research Officers: *D. Dunstan (Leader), M. Lapp, E. Tsang (on leave)*
Research Associates: *S. Adam, B. McAfee, G. Pilate*
Technical Officers: *T. Bethune, C. Bock, J. Malinek, K. Czechowicz (term)*
Summer Student: *N. Beaudoin*
Guest Researchers: *R. Browne , S. Kikcio, D.Lashta , M. Luisdorf, M.Moore, T. Tautorus*

3.3.2 Introduction

The objective of the Project is the development of biotechnology for propagation and improvement of coniferous forest species via provision of superior, elite, or genetically engineered trees.

Canada's forests of the future must contain trees with traits affording high quality wood for lumber, pulp and paper applications. We aim to produce new tree prototypes through cell and tissue culture, and by molecular biology.

In 1989/90 we continued fruitful collaborations with research groups at the Universities of Alberta, Calgary and Saskatchewan, the Pacific Forest Research Centre (Forestry Canada), and the Forest Biotechnology Centre of BC Research Corporation. We received experimental material from such collaborators, as well as from provincial forestry branches in British Columbia and Saskatchewan, and from Weyerhaeuser Canada.

Conifer tree micropropagation remains one of our important activities. Organogenesis of juvenile tissues from western white pine (*Pinus monticola)* has led to effective shoot induction and elongation procedures. Elongated shoots have been rooted and some material has been transplanted, acclimatized and sent to Forestry Canada for evaluation. In work on optimization of plantlet production from somatic embryo cultures of white and black spruces, we regenerated transplantable white spruce plantlets more efficiently using elevated concentrations of abscisic acid.

Somatic embryo suspensions of white and black spruce provided valuable tissue for studies of transient gene expression using electroporation. Transformation experiments used both direct and *Agrobacterium* - mediated methods. Electroporation continued to be used in the evaluation of promoter and gene constructs. Putative transformants have been recovered following biolistic bombardment of somatic embryos.

A major flood at PBI in March 1990 caused the loss of much research material. This included cultures which were part of comprehensive series of lengthy experiments, for assessment of transforming DNA.

3.3.3 Plant Propagation by Organogenesis (M. S. Lapp)

Two main age categories of donor material were investigated, juvenile, and adolescent/adult. The year's work on juvenile tissue is summarized below.

Propagation of western white pine (*Pinus monticola).* Our first objective was to develop procedures for the induction, elongation, multiplication and rooting of shoot derived from mature zygotic embryos. These steps have been accomplished and we are proceeding with their optimization.

Can ectomycorrhizal fungi stimulate rooting in conifers? An *in vitro* system of co-cultivation using de-rooted *Pinus monticola* hypocotyls showed that the presence of three fungi increased rooting over that of control seedlings. *Pisolithus tinctorius* produced more roots per seedling, while seedlings co-cultivated with *Laccaria bicolor* had longer roots. Similar co-cultivation experiments with *Agrobacterium rhizogenes* showed that three wild-type isolates increased rooting, on de-rooted seedlings of *Pinus banksiana* and larch (*Larix laricina).* Rooting of micropropagated *Pinus monticola* shoots was improved when shoots were co-cultivated with *A. rhizogenes.*

Our second objective was to develop procedures for the induction, elongation, multiplication and rooting of adventitious shoots derived from buds from two- and three-year-old seedlings.

Experiments on shoot production and survival using 1988-89 winter buds came to their conclusion in 1989. The most significant factor in production of shoots from buds was the time of year that the explant was removed from the seedling. Thus the optimal medium and benzyladenine concentration varied with the time of year in which the explant was taken. Results with1989-90 winter buds confirmed these observations .

Factors affecting the survival of shoots from buds were assessed. Explant genotype and initial shoot length were the only consistently significant factors. Shoot induction medium and benzyladenine concentration had some effect but this was not consistent. No roots developed on these shoots.

Several attempts were made to establish embryogenic callus cultures from mature embryos of

A mitotic spindle from a somatic embryo of black spruce. T. Tautorus photo.

An acetocarmine stained sample of somatic embryos of black spruce.

western white pine. No embryogenic callus has been obtained but we have been successful in growing long-term non-embryogenic callus. In response to a request from Forestry Canada, callus cultures from six different seed lots were established and challenged with a fungal homogenate, with the resulting cultures frozen for future analysis. Unfortunately the continuing calli of these lines were lost in the March 1990 flood.

The foregoing work involved collaboration with the Pacific Forest Research Centre of Forestry Canada which acted both as a source of seeds and trees and for the planting-out of micropropagated plantlets. (This same group will be one of the major users of this technology).

We also examined micropropagation methods for adult Douglas fir tissues; unlike juvenile material, older conifer tissues show little or no inclination to form new shoots or roots.

3.3.4 Plant Propagation by Embryogenesis (D. I Dunstan)

Our main objective was to optimize recovery of embryo-derived white spruce plantlets competent for transplantation.

Our cell lines had become altered in their ability to regenerate under previously defined conditions. We showed that this was a result of our method of routine subculture, which had been designed to eliminate the week-to-week variability in culture performance. Later, we found that subculture had to be performed subjectively, preferentially selecting certain cell types in order to retain the predominant culture characteristics.

Upon retrieval of the desired characteristics we carried out extensive experiments on optimization of plantlet recovery from somatic embryo cultures, mediated by abscisic acid. High levels of abscisate gave better results than had previously been obtained, permitting a greater developmental regulation, higher "conversion" frequencies, superior germination and better shoot growth. PBI's abscisic acid analog PBI-31 was at least as effective as the natural hormone in these respects.

3.3.5 Plant Improvement by genetic engineering (D. I. Dunstan, E. W. Tsang)

Transgenic technology will have application to those situations for which single genes/enzymes can control phenotypic characteristics, such as genes that could confer increased cold resistance, improved pest repellence, or altered constitution of wood.

Electroporation

Transient gene expression in white spruce. Our immediate objective was to optimize transient gene expression by electroporation. Obtaining stable transformants is a next goal.

Our 1989/90 research built upon results obtained earlier, and included an investigation into the utility of the electroporation technique for cell and molecular biology investigations. We subsequently went on to look at the effects of different promoters on transient CAT and GUS activity in a number of cell lines and species. This showed that use of the tandem repeat of the cauliflower mosaic virus promoter (CaMV 35S-CaMV 35S) resulted in greatly enhanced transient gene expression; this work was done in collaboration with Drs W.L. Crosby and R.S.S. Datla. The conifer transient expression system was also useful in investigations of the regulation of alcohol

dehydrogenase gene (Adh) promoter sequences. This study, done in collaboration with Dr Allen Good, showed that while spruce protoplasts could accurately regulate the Adh promoter from maize, they could not regulate the equivalent promoters from pea or *Arabidopsis*. We also collaborated with Drs Crosby and Datla to demonstrate the efficacy of their alfalfa mosaic virus leader sequence constructs for transient expression in white spruce, tobacco, and maize protoplasts. This sequence, fused to the 35S tandem repeat, increased expression in electroporated white spruce and tobacco protoplasts.

Agrobacterium transformation

The focus of attention was on the use of co-cultivation with maturing, cotyledonary, somatic embryos.

Our aim was to achieve genetic manipulations or modifications using *Agrobacterium*-mediated genetic transformation of white spruce (*Picea glauca*). We investigated the *in vitro* response of organogenetic explants to antibiotics used as selectable markers. The putative transformation noted in 1989 has not been confirmed either for zygotic embryo cultures or for somatic embryo cultures. Mild enzymatic treatment of the suspension cells (somatic embryos) to create "wound sites" did allow the attachment of bacteria, but without transformation. Similarly the use of feeder layers failed to give transformation. A considerable difficulty was the eradication of the infecting bacteria. An intron-containing GUS gene construct which prohibits GUS gene expression in *Agrobacterium*, was not spliced in the conifer system.

3.3.6 Extramural Interactions

University links

Formal collaborations exist between the Project and the Universities of Calgary (Biological Sciences - Profs. Thorpe and Moloney) and Saskatchewan (Biology - Profs. Fowke and Steeves). The collaboration with Profs.Thorpe/Moloney, and that with Prof. Fowke, were assisted by strategic NSERC grants. Guests Robin Browne and Tom Tautorus were graduate students of the University of Saskatchewan.

A collaboration with Prof. Fowke and Dr. Stephen Attree (U. of Saskatchewan) led to the first report of plantlet regeneration from conifer protoplasts in 1989. An ultrastructural investigation of white spruce protoplasts was carried out to determine the occurrence of microtubules, structures typically involved with cell wall organization and cell division. Work also progressed with the production of plantlets from somatic embryos of *Picea mariana* and *Picea glauca*, showing that these two spruce species were dissimilar in their growth performances after transplantation.

Work was extended to black spruce (*Picea mariana)*, and protoplast regeneration in jack pine *(Pinus banksiana)* through the thesis research of guest researcher Tom Tautorus, a Ph.D graduate student who is jointly supervised by Prof. Fowke and David Dunstan, with assistance from Dr. Attree. This research developed methodology for the production of somatic embryo callus and suspension cultures, from mature embryos in seed that had been stored for many years, and from immature zygotic embryos. Suspensions were then used as a source of tissue for protoplasts. Subsequently, procedures were developed for protoplast culture and for their regeneration to embryos. Electroporation research with these species characterized the requirements for transient gene expression with GUS and CAT reporter genes.

Private Sector links

Formal collaboration existed with AgriForest Technologies Ltd., Kelowna, B.C. The Project is also working with the Forest Biotechnology Centre (BC Research). Under this arrangement three employees of BC Research are in our laboratories as guest researchers. The contract commenced in October 1989 and started work in January 1990.

We received valuable research material, provided free of charge, from: Weyerhaeuser Canada, B.C. Ministry of Forests and Lands, Saskatchewan Parks and Renewable Resources, Forestry Canada (Pacific Forest Research Centre), and other organizations.

4. Gene Technology Section

Head: Dr. W. L. Crosby (to September 1989)
Dr. John D. Mahon (from October 1989)

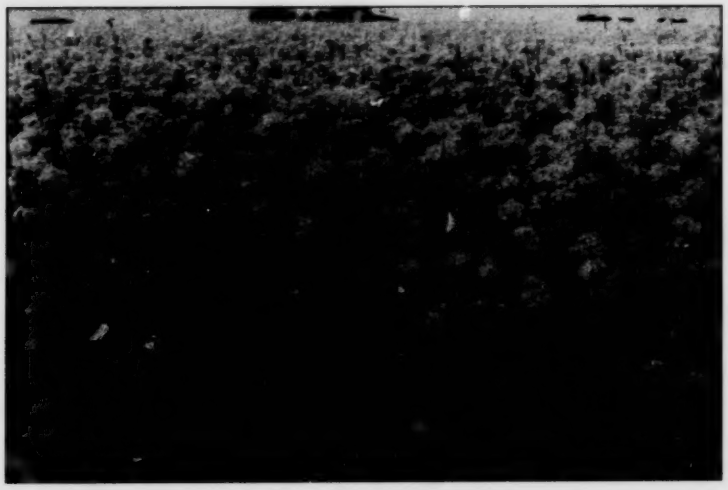
4.1 Overview

PBI's Gene Technology Section completed its fifth year of operation in March 1990, having been established only in 1985. The Section, now up to its full strength of thirty NRC staff, also accommodates nearly twenty non-NRC guests in its laboratories.

All three Section projects are concerned with the operation and control of genes in plants. The project on stress tolerance genes examines particularly the molecular responses of plants that can lead to osmo-tolerance (salt tolerance) and resistance to fungal attack. Regulation of genes in plants focuses specifically on mechanisms by which gene action is promoted or constrained in plants. The project on legume biotechnology develops genetic technologies to improve grain legume species important to Canada.

Recombinant DNA techniques are central to this Section's interests. Accordingly, excellent facilities for study of these have been built up since 1985; these now include units for automated DNA strand synthesis, amplification and sequencing.

For all Section groups, 1989/90 proved a highly productive year, and a time of modest growth and specialization. The former group "Plant Molecular Genetics" gave rise during the year to two projects based on its main activites: stress tolerance genes and regulation of gene expression in plants.



Canola is a common target of genetic engineering experimentation at PBI.

4.2 Stress Tolerance Genes Project

4.2.1 Personnel:

Research Officers: *G. Selvaraj (Leader), L.E. Pelcher, J. Taylor*
Research Associates: *L. Adam, A.A.K. Hussain, V. Morales, S. Pedras, G. Penner.*
Technical Officers: *C. Barber, I. Borgmann, R. Hirji, B. Panchuk, D.Schwab*
Guest Researchers: *L. Boyd, R. Jain, K. Rozwadowski, A. Woytowich*
Summer Student: *Susan Liu*

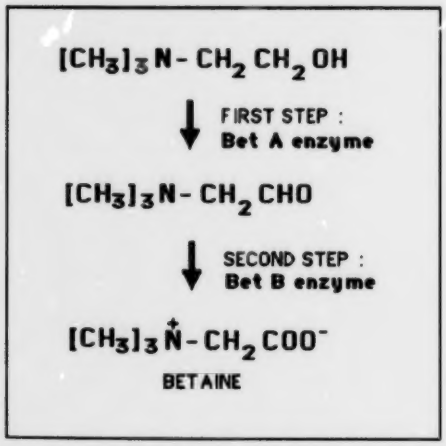
4.2.2 Introduction:

This Project has been operational since August 1989, and has three research laboratories along with a unit for DNA sequencing, amplification and oligonucleotide synthesis. During 1989/90 we continued to pursue several closely related objectives in the area of gene products involved in plant stress responses. Our objectives were: (1) to find genes and gene products which are involved in cellular adaptation to osmotic stress; (2) to describe the biochemical responses of plants to fungal pathogens; (3) to construct transformation vectors for plants and fungi, and reporter gene modules suitable for retrieving promoters in plants; (4) to provide DNA services to the Institute.

4.2.3 Molecular Biology of Osmotolerance (G. Selvaraj)

Our study concerned the biochemical basis of osmotolerance ("salt-tolerance") at the cellular level, focussed on the role of trimethylglycine in adaptation to osmotic stress, and on the genes and gene products involved in osmotolerance.

Betaine (trimethylglycine) accumulates in many organisms and protects them against osmotic stress. Genetic and biochemical evidence confirms the osmoprotective role of betaine in bacterial systems, and some salt- tolerant plants appear to accumulate betaine as an adaptive response, thus providing a basis to manipulate this trait in plants. During 1989/90 we isolated and characterized bacterial genes involved in betaine biosynthesis. Betaine synthesis from its precursor choline differs in plants and bacteria only in the type of oxidation reaction in the first of the two steps. Thus bacterial genes should be useful in plants.



Formation of betaine from choline requires two steps.

From *Escherichia coli* we cloned genes encoding a membrane-bound choline dehydrogenase (*bet* A) and a soluble betaine aldehyde dehydrogenase (*bet* B). A DNA fragment containing these genes was sequenced and the open reading frames corresponding to the genes, were delineated. The *bet* A gene probably encodes a 556-unit polypeptide. The *bet* B gene product was ca. 40% identical with the primary structure of known aldehyde dehydrogenases of fungal, rat and human origin. (If biochemically similar amino acid substitution was included in the comparison, the similarity increased to ca. 75%) placing the enzyme among the most highly conserved enzymes in evolution.

Plant Cells under Osmotic Stress

Plant cells respond to salinity in their environments by altering their patterns of gene expression. Some of these can be useful in regulating genes encoding betaine biosynthesis. We therefore screened proteins in *Kochia scoparia*, a salt tolerant plant found to survive in saline patches.

We identified four salt-induced polypeptides in *Kochia* roots, with molecular weights ranging from 22 000 to 66 000. But attempts to purify quantities for amino acid sequencing have not yet been successful. Two salt-inducible proteins were identified in *Kochia* leaf extracts.

4.2.4 Molecular Biology of Plant - Pathogen Interactions (J. L. Taylor)

This work concerning identification of plant responses to fungal infection aims to provide an understanding of the molecular basis of fungal pathogenesis. Plants can potentially thwart microbial pathogens by synthesizing small antibiotic substances (phytoalexins), cell wall degrading enzymes (e.g., chitinases and glucanases) and lectins that inhibit fungal cell wall synthesis.

Our work has dealt with the defense strategies of plants and the molecular mechanisms of fungal virulence. Successful infection by a pathogen is contingent on overcoming a plant's defense strategies and estab-

Dr. Janet Taylor examines a culture plate in a stress tolerance experiment.

lishing the conditions that allow the pathogen to live on within the plant. Our work, done in collaboration with staff at Agriculture Canada and at the University of Manitoba, focused on the mechanisms by which *Leptosphaeria maculans* causes blackleg disease in canola.

Virulent forms of the blackleg fungus possess the ability to destroy phytoalexins. We observed that the fungus catabolized at least one of the Brassica phytoalexins, and we identified several catabolic products derived from the phytoalexin.

As part of our research into the genes involved in the blackleg disease process, we are developing a fungal transformation system. Two transformation vectors suitable for cloning and re-introduction of gene constructs into fungi of agricultural importance were designed and constructed. An efficient transformation system is needed for *L. maculans*, and toward this end, a number of fungal mutants have been isolated.

Five restriction fragments representing portions of the middle repetitive DNA component of the *L. maculans* genome were cloned and sequenced. The hybridization patterns with these as probes clearly distinguished virulent isolates from weakly virulent isolates. Chromosomes of *L. maculans* were isolated by Transverse Alternating Field Electrophoresis (TAFE); this required the development of new methodology for isolating whole chromosomes and for electrophoretically separating them by TAFE. Analysis of virulent and weakly virulent isolates showed vast differences both in chromosome number and size.

4.2.5 Synthesis and Sequencing of DNA

(L. E. Pelcher)

The DNA Services Unit went into full operation at the beginning of 1989/90, with facilities to synthesize and purify DNA single strands (oligos). These oligos have been used in applications in many laboratories in the Institute and beyond. This unit also is a central facility for automated DNA sequence determination and DNA amplification, and has been involved in development of these technologies.

In 1989/90 the unit produced 550 oligos (average length 25 bases) for 30 researchers at PBI. Chemical phosphorylation of 5' ends was introduced as an optional feature, and this has improved the efficiency of applications involving oligos with 5' phosphoryl groups. Other service improvements in 89/90 included provision of modified thymidine with a special linker arm for immobilizing alkaline phosphatase enzymes. A high performance liquid chromatogaphy system, installed in January 1990, has improved purity of oligos and has increased throughputs in synthesis and purification. Our thermal cyclers have been used to derive a number of polymerase chain reaction products, which in turn have been used to synthesize DNA templates, genes and gene segments for a variety of applications.

Almost 30 000 bases of DNA have been sequenced since setting up the sequencing facility, and last year's acquisition of an automated sequencer has improved the throughput. The reaction conditions were optimized to suit the primer fluorescence-based chemistry, and we were able to derive reproducible and reliable sequence information. Significant advances in direct sequencing of amplified DNA have thus been achieved.

4.2.6 Extramural Interactions

Two President's Fund awards were held during the year, and collaborations with researchers at Agriculture Canada continued. Considerable time was devoted to graduate student research guests from the University of Saskatchewan, to work with colleagues at the University of Manitoba, and to assisting non-project users of our DNA services.

4.3 Regulation of Gene Expression in Plants Project

4.3.1 Personnel:

Research Officers: *S. M. Hemmingsen (Leader), W. L. Crosby*
Research Associates: *F.Bekkaoui, L.Cloney, R. Datla, A. Good, L. Klimczak*
Technical Officers: *J. Condie, J. Hammerlindl, D. Kneeshaw*
Guest Researchers: *I. Babic, M. Balisubramanian, C. Bennett, G. Feist*
Summer Student: *G. Samuel, P. David*

4.3.2 Introduction

In nature, plant genes are regulated by means not yet fully understood. Control of gene action in the context of improving crop performance is a central goal in biotechnology, and is especially important in creating biochemically efficient transgenic systems.

An objective of this project, separately established in August 1989, is to discover how plant genes can be controlled after transcription and after translation. The demonstration of regulated transgenic systems is also an aim.

4.3.3 Molecular Chaperones

(S. M. Hemmingsen)

The discovery of the essential roles played by molecular chaperones in gene expression has challenged the principle that all polypeptides spontaneously "self-assemble". The level of research activity in this area has grown very rapidly since publication of our findings in 1988.

We produced three articles reviewing aspects of molecular chaperones which appeared during this year, and we also submitted a manuscript describing the cDNA sequences of *B. napus* Chaperonin - 60 alpha and beta polypeptides and our analysis of these sequences. We pursued three approaches to investigating the structural and functional roles of the alpha and beta polypeptides in the chaperonin-60 protein and in chaperonin function: antisense; antibodies; and complementation of chaperonin function in *E. coli.*

In our antisense studies we have transformed *Arabidopsis thaliana* plants with antisense alpha and antisense beta constructs and are now assessing the phenotypes of these plants. We have produced polyclonal antibodies against alpha and beta polypeptides produced in *E. coli* and against alpha specific and beta specific conjugated oligo-peptides. These antibodies appear specific for each polypeptide or for specific domains of each polypeptide; they could lead to understanding of the subunit structure of chloroplast Chaperonin-60.

Via complementation we made extensive efforts to achieve the assembly of plant rubisco enzyme in *E. coli* based on our understanding of the roles of molecular chaperones and exploiting our lead in having been the first laboratory to isolate alpha and beta cDNA clones. These experiments have not yet produced plant rubisco activity in *E. coli* (although they have led to further insights into chaperonin function) and were set in abeyance pending identification of either the eukaryotic chaperonin-10 homolog or the chaperonin-60 species involved in rubisco gene expression.

Technical officer Colleen Bennett protects experimental plants against cross pollination in the Transgenic Plant Centre.

4.3.4 Bifunctional Fusion Genes and Recovery of Plant Regulatory DNA Elements (W. L. Crosby)

The project has developed and characterized a bifunctional fusion gene between *E. coli* beta-glucuronidase (GUS) and neomycin phosphotransferase II (NPT). This gene offers advantages for simultaneous selection and biochemical screening of transgenic plants, but has been taken to a second level of development as an *in vivo* probe for the identification of plant regulatory elements.

During 1989/90 we succeeded in identifying tissue-specific and organ-specific promoters in tobacco using synthetic, T-DNA-mediated random 'transposition' of promoterless fusion gene constructs into leaf-disc explants. We have recovered several of these regulatory regions, thus making them suitable for use in tissue-specific heterologous expression.

In addition to the GUS/NPT fusion gene, a fusion gene between chloramphenicol acetyltransferase (CAT) and NPT is under development for use in eukaryotic systems where GUS may not be a suitable marker.

4.3.5 Viral Transcribed Non-Translated Leader Sequences (W. L. Crosby)

To address the need for enhanced heterologous expression of several genes, we explored the fundamental role of 5' transcribed, non-translated mRNA leader sequences in raising expression of foreign genes in various plants. In this study, we assessed leaders from diverse sources including plant viruses, chloroplast ribosomal proteins and seed storage proteins. In electroporation experiments the leader from alfalfa mosaic virus RNA4 had a 4- to 6-fold enhancing effect with both GUS and CAT marker genes. Inserted in combination with a high-activity 35S-tandem promoter, the leader/promoter combination delivered up to 60 times the level of 35S expression in tobacco, and 20 times in electroporation experiments.

4.3.6 Molecular Regulation of the Acetolactate Synthase Gene Family in *B. napus* (W. L. Crosby)

Two members of the acetolactate synthase (ALS) gene family from *B. napus* had earlier been isolated and sequenced. This year, the group succeeded in demonstrating a herbicide-resistance phenotype of site-directed mutants of the Brassica gene, and has indications that the gene is preferentially expressed in developing flowers and embryos. Tobacco plants transgenic for the intact *B. napus* gene showed low levels of herbicide resistance, whereas heterologous constructs employing our 35S-alfalfa mosaic virus construct expressed high-level tolerance to sulfonylureas.

From sequence information, the group identified a 14-base motif which is precisely conserved in the *B. napus* and *Arabidopsis* upstream regions. This sequence proved essential for gene function. Duplication of this putative enhancer element in the *Arabidopsis* gene resulted in higher levels of gene activity and herbicide tolerance in transgenic tobacco.

Taken all together, the year's progress has brought us close to tissue specific regulation of at least some member of the *B. napus* ALS gene family. In addition, our identified enhancer-like sequences provide an approach to analyze the sequence-specific interaction of an ALS upstream sequence with trans-acting factors.

4.3.7 Extramural Interactions

Although our work has been described as "basic", we performed some research under industrial sponsorship (Philom Bios Inc. and PARTEQ/Queens University), and have continued collaborations with a large number of university groups, some of which have been mentioned above. We were participants in NSERC-assisted studies, and have held NRC President's Awards for the year's research.

Research collaborators during the year included Dr. Maurice Moloney (U. of Calgary) and Dr. R. Blackshaw (Agriculture Canada/Lethbridge), R. J. Ellis (Warwick Univ.), and groups at Queen's University.

Researchers in our project made an extraordinarily large number of invited talks and presentations including talks at the University of Basel, U. of Warwick (U.K.), US Department of Agriculture, Yale University, European Molecular Biology Laboratory, DuPont (Wilmington), Pakistan Agricultural Research Council, and the Canadian Society for Plant Molecular Biology.

4.4 Legume Biotechnology Project

4.4.1 Personnel

Research Officers: *J. Mahon (Leader), S. Hobbs, L. M. Nelson*
Technical Officers: *M. Anderson, D. Baliski, C. DeLong, C. Knapp*
Research Associates: *J. Jackson (to Nov. 1989), H. Rempel (from June 1989), M. Jordan (from Sept. 1989)*
Guest Researchers: *S. Sprout, P. Kpodar, D. Feindel, M. Luisdorf, C. van Kessel, S. Grayston, D. Knight, N. Rueker, D. Chenier.*
Summer Students: *D. Durnford, N. Ruecker, D. Chenier*

4.4.2 Introduction

The overall objective of the project is to promote the genetic improvement of Canadian grain legume crops through the application of biotechnology. As we have identified the inability to introduce foreign genes into plants of these important agricultural species as the major obstacle, the project continued to emphasize the development of transgenic technology for legumes during 1989/90. After the previous year's demonstration of successful gene transfer to pea by wild-type *Agrobacterium tumefaciens*, we concentrated on the development of a transformation system based on the introduction of genes by disarmed *Agrobacterium* vectors and on an improved method of regenerating pea plants from tissue explants which are amenable to infection by *Agrobacterium*. Other research included a major experiment on the control of gene expression in transgenic plants growing in different environments. Work on plant growth promoting rhizobacteria was transferred to the MicroBio Rhizogen Corp. and collaborative work funded by IRAP, was initiated in February 1990.

In June, Dr. Hans Rempel was added to the group to initiate work on *Agrobacterium* virulence in pea and to develop improved *Agrobacterium*-based vectors for transformation. In November, Dr. Mark Jordan replaced Dr. Jennie Jackson on the tissue culture area and to integrate the regeneration and transformation protocols.

4.4.3 Transgenic Technology for Pea
(J. D. Mahon, L. M. Nelson)

Plant Regeneration Systems

The regeneration of pea plants from cells and tissues by reported methods is slow and variable in efficiency for different pea types. We have overcome these problems by developing a greatly improved regeneration system using cotyledonary node explants. All such explants from six different pea genotypes produced an average of six to ten buds when cultured with benzylaminopurine, as compared to an average of fewer than [illegible] per explant after 56 days with other explants. The buds formed in the superficial layers of the explants which would be amenable to *Agrobacterium* or particle bombardment and developed into shoots which could be readily rooted to produce plants.

Gene Transfer and Detection Technology

In order to carry out transformation studies with a recalcitrant plant species, selectable and reporter genes must be tested for their ability to distinguish transformed from untransformed tissues, and to be combined with regulatory sequences which work in the target species. To ensure that such genes are effectively expressed in pea tissues, we electroporated binary vectors containing the GUS marker gene with several reported constitutive regulatory sequences (obtained from the Gene Regulation Project) into pea protoplasts. Although the GUS activity differed among pea genotypes, protoplasts from leaf and root tissues were similar. The nopaline synthase and CaMV35S promotoers gave similar levels of expression, but the tandem CaMV35S-CaMV35S promoter was superior and the inclusion of an Alfalfa Mosaic Virus mRNA leader sequence resulted in much higher expression with all promoters.

We compared the selectivity of genes conferring kanamycin and hygromycin resistance by constructing a binary vector containing both the neomycin and the hygromycin phosphotransferase genes. We inserted it into 3 different disarmed *Agrobacterium* strains. We also developed a pea epicotyl system that produces transformed callus tissue at high frequency within eight weeks after *Agrobacterium* infection, and used it to test these vectors. Hygromycin was superior to kanamycin as a selective agent in pea tissues, killing the untransformed tissues within three weeks, and one disarmed A281 strain was most virulent in pea. This agreed with the results of challenging plants or leaflet explants with thirteen wild-type strains, of which only A281 and one other showed high virulence. This elevated virulence of A281 has been attributed to genes in the *vir* region of its plasmid. Transferring this region to less virulent strains increased infectivity while addition of copies to A281 amplified *vir* region induction. As an approach to increasing virulence in pea, subclones of the *vir* regions from A281 were inserted into a high-copy number plasmid to be transferred into *Agrobacterium* strains (including A281).

The GUS gene is useful in transformation studies because it offers a sensitive measure of gene expression and a stain which can identify the tissues

in which it is expressed. This ability to see which cells have been transformed is very important for the production of transgenic legumes, as the *Agrobacterium* vectors must infect cells in a localized area from which plants can be regenerated. However, false positives can arise from expression of the GUS gene by residual *Agrobacterium* cells. To overcome this, we constructed a binary vector which combines selective markers and a GUS construct (from Dr. G. Vancanneyt, Berlin), which is not expressed by bacteria because it contains a plant intron.

As an alternative to binary *Agrobacterium* vectors, a set of vectors based on the Monsanto split-end system were constructed. These contained either the GUS or the chloramphenicol acetyl transferase (CAT) marker genes combined with four different plant promoters. We tested them with tobacco and with our pea callus system.

Transformation in Cotyledonary Node Explants

Our rapidly regenerating cotyledonary node system has been the major target for the production of transgenic pea. Tissues were co-cultivated with disarmed *Agrobacterium* strains harbouring a binary vector carrying NPT and GUS and allowed to regenerate shoots in the presence of kanamycin. Regenerated shoots produced roots in kanamycin-containing medium. More than 450 of these plants were successfully transferred to soil; some produced seed. Using the newer binary vectors described above, small buds produced on cotyledonary node tissues survived hygromycin selection. Moreover, within two weeks of co-cultivation with the intron-containing GUS gene, we observed organized structures with positive histochemical evidence of GUS activity in all cells.

4.4.4 Control of Gene Expression in Transgenic Plants (S. L. Hobbs)

The effective use of biotechnology to genetically improve crop species requires not only a transformation system and agronomically beneficial genes, but also an understanding of how the expression of genes is controlled in transgenic plants. Often, foreign genes can vary greatly in their expression for no evident reason. To examine the causes of this, we observed inter- and intra-transformant variability in GUS expression in transgenic tobacco plants and their progeny grown in three environments, including the field. (The experiments included PBI's first field test of transgenic plant material and thus established for the Institute an understanding of the procedures.) Field expression of a single gene construct proved very similar in the field to that observed in controlled environments.

4.4.5 Extramural Interactions

Major collaborations began with MicroBio Rhizogen Inc. on plant growth promoters, and earlier collaborative work with the Crop Development Centre continued in legume types for breeding programs. Funded work was set up via a contract with the Saskatchewan Agriculture Development Fund, for identification of *Ascochyta* resistance genes (A. Slinkard, collaborator).

Invited talks were given by project members at ten North American conferences and symposia, and eight Canadian companies were briefed on the group's progress in 88/89. Contacts were continued with Agriculture Canada research stations and with several Canadian university groups.

5. Biological Chemistry Section

Head: Dr. E.W. Underhill (through February 1990),
Dr. S.R. Abrams (from March 1990)

5.1 Overview

This Section provides a strong "molecular" element in the Institute's molecular biology activities. The eleven component laboratories have excellent facilities for chemical synthesis, molecular separation and analysis, and for research on polypeptide characterization and manipulation.

The Institute has a long-standing record of excellence in phytochemistry. During the year this was manifested in the work of the three major project groups of the Section. Under the leadership of Dr. Suzanne Abrams, new synthetic plant hormones discovered at PBI have been subjected to extensive biological tests, with very gratifying results. This work on abscisic acids was upgraded to full project status during the year.

PBI's efforts to develop biotechnological means of enhancing the value of plant products began in 1987. This line of work, having proved productive and promising, became a full-scale project on seed oil modification during 1989, under the leadership first of Dr. E.W. Underhill, then of Dr. S.L. MacKenzie. Its aims include development of prototype transgenic plants with high contents of industrially important fatty acids.

Long-active work on plant cell metabolism and the use of plant cells in artificial culture as production systems for high-value phytochemicals continued in 1989/90 led by Dr. W.G.W. Kurz. Some industry-laboratory joint projects came to an end this year, but core work on control of secondary product formation was carried on.

This Section continued to host large numbers of guest researchers in 1989/90, and new physical space for industry collaborations was provided by redevelopment in older areas. The industrial "incubator" laboratories located in the Section remained active through the year.

5.2 Bio-Organic Chemistry Project

5.2.1 Personnel:

Research Officers: *S. R. Abrams (Leader), T. Mazurek, G. P. Slater*
Research Associates: *L.A.K. Nelson, N. Lamb*
Technical Officers: *A. Shaw, L. Friesen, L.R. Hogge, D. Olson, L. Hoffman*
Guest Workers: *G. Abrams, C. Craig, C. Hampson*
Summer student: *K. Carrusca, G. Cleghorn*

5.2.2 Introduction

The overall objective of the abscisic acid research effort at PBI is to control growth and development of plants by manipulating processes regulated by this hormone and its analogs. Abscisic acid (ABA) is involved in many phases of plant development including germination, transpiration, growth, dormancy, senescence, and resistance to environmental stresses. A major goal of the project is to determine the biological role of ABA-related individual compounds so that plant productivity can be improved by targetted genetic modifications. As well, novel ABA-related plant growth regulators are being developed that have practical application in forestry, agriculture, processing of agricultural products, and as tools for plant tissue culture research.

Our work on protection of crops from insects has aimed at identifying volatiles produced by brown mustard (*Brassica juncea)* which attract the diamondback moth, a serious pest of this potential crop species.

5.2.3 Abscisic acid metabolites and their biological activity (S. R. Abrams)

Substantial progress was made in1989/90 in establishing the nature and biological role of compounds in the ABA pathway. Using our newly developed techniques for separating optical isomers of ABA, and our synthetic methods to produce optically pure analogs, we found striking differences in the biological activity and metabolism of optical isomers of ABA and analogs. The program for screening analogs for germination, transpiration and cold tolerance has concluded, and specific analogs have been identified that have practical application as plant growth regulators.

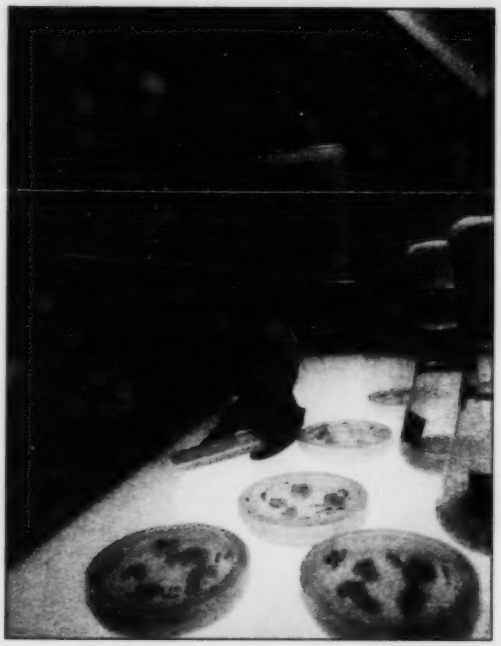
Parts of this work were carried out in collaboration with Professor L.V. Gusta, M.T.J. Reaney and A.J. Robertson (U. of Saskatchewan). Bromegrass cell suspension cultures were used as a model system for studying the mechanism of cold tolerance in plants. ABA, supplied to cells increased the freezing tolerance from -8 to -40°C after 7 days. We used this model system to determine the ABA metabolic pathway in plants.

Racemic ABA-related compounds abscisyl alcohol, abscisyl aldehyde, and phaseic acid were synthesized. These chemicals, along with ABA, were fed to bromegrass cells. After treatment for 7 days, the culture filtrates were examined for metabolites. The pathway deduced from these results is shown below.

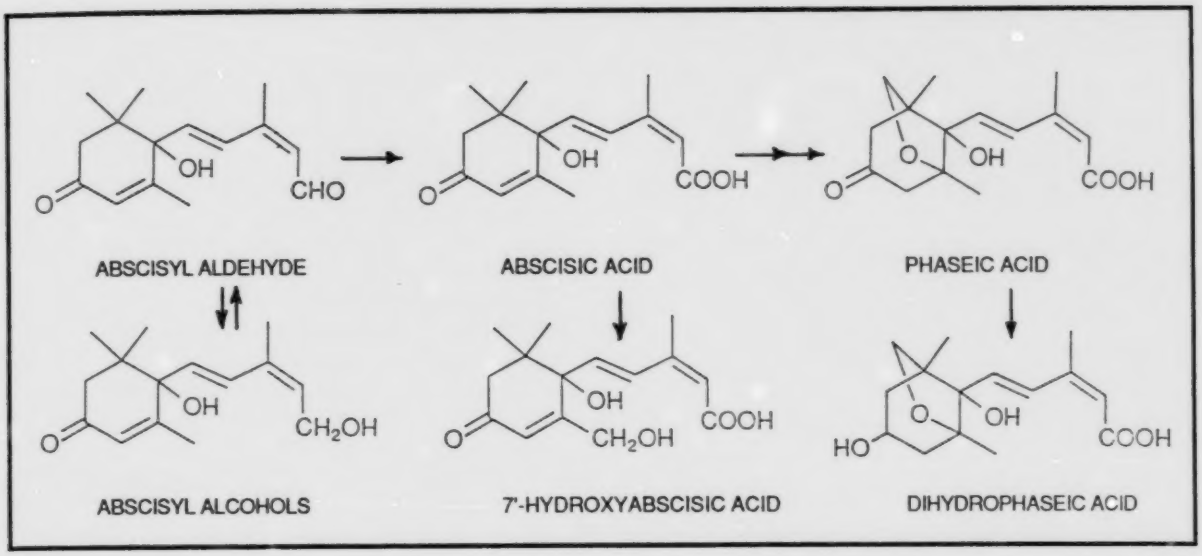
Abscisyl aldehyde, ABA, and phaseic acid were all transformed to dihydrophaseic acid; ABA is also converted to 7'-hydroxyabscisic acid. Abscisyl alcohol was isomerized to the 2-trans form, presumably through reversible oxidation to the aldehyde. Although ABA itself conferred the greatest freezing tolerance to the cells, abscisyl aldehyde was moderately effective in cold tolerance and in growth inhibition, likely due to its conversion to ABA. Phaseic acid had no effect on freezing tolerance and growth inhibition, so the metabolism of ABA to phaseic acid is not essential for freezing tolerance.

Activity of optical isomers in freezing tolerance

We were able for the first time to obtain milligram quantities of unnatural (-)-ABA by resolving racemic methyl abscisate on a preparative chiral column. (+)-ABA can be obtained in quantity as a fungal metabolite, but the unnatural ABA has not



Technical officer Laurie Friesen checks callus growth in abscisic acid experiment.



Formation and metabolism of abscisic acid in plants.

been found in nature. Our (-)-ABA, tested in cold tolerance assays induced a small degree of freezing tolerance (-19ºC vs. the untreated control -11°C).

Our analog PBI-38 mimics abscisic acid, but because it is lacking an essential double bond, cannot be metabolized by plant cells to phaseic acid. This compound proved to be a tool for separating the effects of ABA from those of its metabolites. In bromegrass PBI-38 substituted for ABA, inducing a freezing tolerance of -37°C as opposed to -40°C with ABA. In tests of optically active forms of this compound, the isomer with the same absolute configuration at C-1' as natural (+)-ABA imparted freezing tolerance (-34.5°C), while the form which resembles (-)-ABA was inactive. This effect confirmed that abscisate and not phaseate is the hormone.

PBI-38 has application as a plant growth regulator in plant tissue culture regimes, and in conifer seedling conditioning. This analog has already been identified as a potent antitranspirant in black spruce seedlings, and is now being tested industrially for this.

Screening of ABA Analogs

A study of the relationship between antitranspirant activity and structural modifications to the ABA molecule has been completed. Analogs altered in oxidation levels at C-1 and in unsaturation patterns at C-2,3, C-4,5, and C-2',3' were applied to tomato plants before transplanting. Effects on transpiration and water content were measured in the field and in growth chamber experiments. One analog prevented wilting, and decreased time to flower by 5 days.

Effects of the 32 analogs on germination of cress and barley were also measured. Biogenetic precursors to ABA were found to be more potent inhibitors than ABA. The inhibitory effects of abscisic alcohol and aldehyde could be reversed by brief heat treatment of the seeds, but not by water washing.

Biological activity of phaseic acid in barley

This was a collaboration with Professor R.D. Hill, (U. of Manitoba). A study of ABA and phaseic acid in barley embryos showed that both compounds promoted the de novo synthesis of two ABA-inducible proteins, an alpha-amylase inhibitor and barley germ agglutinin. ABA levels in phaseic acid-treated tissues, were not significantly higher than in ungerminated controls, so it is not yet known whether the phaseic acid is acting directly or by feedback inhibition.

5.2.4 Protection of Crops From Insect Pests (G. Slater)

Brassicas are subject to attack by several insect pests such as the diamondback moth. Control and damage in a 1988 infestation amounted in Canada to $25 million. This moth's preference for crucifers and its nocturnal habits indicate that plant odours are involved in locating acceptable hosts. Our objective was to identify the molecules responsible. We analyzed volatiles from plant homogenates and made a detailed study of insect behaviour. This resulted in development of two bioassays, one based on the attraction of female moths, the other on measurement of the voltage induced in an insect's antenna using an electroantennogram. Although the total volatiles were antennally-active, separations showed that activity was due only to one compoud, allylisothiocyanate.

We examined several cruciferous hosts of this insect. Volatiles collected from *B. napus* and *B. juncea* seedlings were essentially identical, except for the allyl-isothiocyanate produced by the latter plant. Preliminary separation by gas chromatograph, as before, gave the same distribution of bioactivity. This fact should help simplify breeding experiments to remove these components. Moths responded positively to seedlings of *B. juncea* and *B. napus* but weakly to *Sinapis alba* and not at all to *Vicia faba*, a non-crucifer. In spite of its weak attraction, *S. alba* proved to be as good a host as *B. juncea*. Stinkweed (*Thlaspi arvense*) was also tested as a host since field observations indicate that it is not attacked by pests. In a no choice situation *P. xylostella* laid eggs on this weed and larvae developed into mature adults indicating the plant is not toxic to the moths. Stinkweed contained mainly allyl isothiocyanate and a small amount of allylthioeynate.

5.2.5 Analytical Chemistry Laboratories

Mass spectrometry

The mass spectrometry facility, a service to the Institute, has three instruments. Our VG 70-250 Hybrid (purchased in 1989) is used to perform mass spectrometric analyses of complex mixtures, giving high resolution, accurate mass data for individual components. The Finnigan 4500 (purchased in 1978) is a GC/MS low resolution instrument used for routine analyses. The Finnigan 3300 (purchased in 1974) has limited use for thermospray experiments.

A total of 1450 analyses were performed in this year, about a quarter of which were for extramural users.

Development work continued on packed capillary fused silica column continuous flow fast atom bombardment. To date, efficient 20 cm columns (10,000 theoretical plates) have been packed in our laboratory and a special ultraviolet detection cell has been designed to handle the low flow rates generated by these columns.

NMR Laboratory

The nuclear magnetic resonance spectrometry facility is also operated as a service for the Institute. The major instrument is a Bruker 360 MHz wide-bore machine. Analyses were performed on four different nuclei: 1H, 13C, 31P and 15N, in living plant cell systems and in solution for normal high resolution structural information. In 1989/90 the facility was heavily used with a total of 1286 spectra recorded.

5.2.6 Extramural Interactions

Many extramural activities went on this year, mainly in the abscisic acid field. A funded project with Prairie Malt Ltd (1990-1992) began to improve malt quality of barley via ABA technology, while a second project with Balco Canfor Reforestation (1988-1990) examined ABA-conditioning of conifer seedlings. Tissue culture applications of ABA were probed in collaboration with Monsanto (St. Louis) and Profs. B. D. McKersie and P.K. Saxena at Guelph University.

Resources from NSERC awards to L. V. Gusta and to M. D. Devine supported collaborations with these scientists. Joint work involving germination control was also carried out with groups at Agriculture Canada and the US Department of Agriculture. R. D. Hill (U. of Manitoba) participated in studies of ABA regulation of gene expression in barley.

On the crop protection side, collaborations continued with the Crop Development Centre of the U. of Saskatchewan and through graduate student placements in our laboratories.

5.3 Seed Oil Modification Project

5.3.1. Personnel

Research Officers: *E.W. Underhill (Leader to 28 Feb 1990), S. L. MacKenzie (Leader from 1 Mar 1990), J.W.D. Grootwassink, D.C. Taylor*
Technical Officers: *D. Barton , E. M. Giblin, A. D. Kolenovsky, D.W. Reed, D.M. Tenaschuk*
Research Associates: *J. Jain, L. Kunst, R. Martel, L. Thomson*
Guest Researchers: *J. King, D. Williams, J. Nowak, G. Haughn, C. Leier, K.. Pivnick, B. Manns, M. Martin.*
Summer Student: *K. Rioux*

5.3.2. Introduction

The goal of our project is the development, through recombinant DNA technology, of new plants capable of producing specifically-designed seed oils which cannot be achieved through current plant breeding techniques. This genetic engineering can proceed only from an understanding of the regulation of lipid synthesis. Knowledge of the properties and regulation of specific enzymes which synthesize, desaturate and elongate fatty acids, and assemble them into storage lipids.

The specific objectives of the project are the genetic modification of rapeseed *(Brassica napus L.*) for increased oil content, and for seed oils very high in erucic acid and ricinoleic acid. These fatty acids have important industrial uses.

Although canola is primarily an oil-based crop, other components of the seed which can affect the viability of the crop and the quality of the oil must also be considered. A general method for suppression of mustard oils (glucosinolates) in cruciferous plants is also one of our goals.

Our research strategies for increasing oil content involve acetyl CoA carboxylase (ACC), a key enzyme regulating fatty acid synthesis. The production of engineered triacylglycerols (TAGs) has involved enzyme isolation and characterization and molecular genetic analysis of mutants. Both approaches can lead to the genes.

Brassica species lack enzymes for inserting erucic acid into the middle position on the glycerol backbone. Enzymes for this exist in nasturtium and meadowfoam, and these are sources of genes to transform *B. napus*.

Enzymes which synthesize erucic acid and ricinoleic acid were studied using *Arabidopsis thaliana* mutants. Mutant selection has also been used in our attempts to reach zero-glucosinolate crucifers.

Dr. David Taylor in one of PBI Organic Chemistry laboratories.

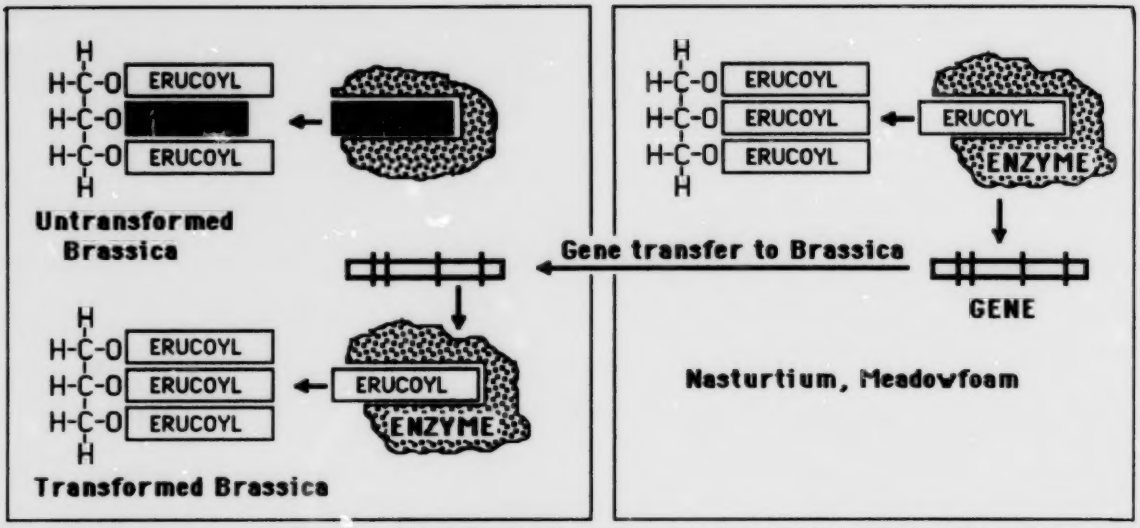
5.3.3 Regulation of Lipid Synthesis

(S. L. MacKenzie)

Acetyl coenzyme A carboxylase (ACC) is a main regulating enzyme in lipid synthesis. Its action controls the amount of initial synthesis of fatty acids which occurs in cells. Our interest in this enzyme stemmed from its potential to enhance the rate of lipid synthesis in oilseeds. Much of our research in 1989/90 was involved with methods for manipulating this idfficult enzyme, and with characterizing the enzyme itself.

We developed a new process for isolating ACC enzyme from plants, using ammonium sulfate precipitation, gel filtration, ion exchange and affinity chromatography. ACC partially purified from developing canola seed and canola microspore-derived embryo cultures was a homo dimer with a subunit size of 220 Kd. Good ACC activities were also detected in extracts from *Arabidopsis thaliana* seedlings.

A polyclonal antibody was raised to barley ACC for screening canola and *A. thaliana* cDNA expression libraries.

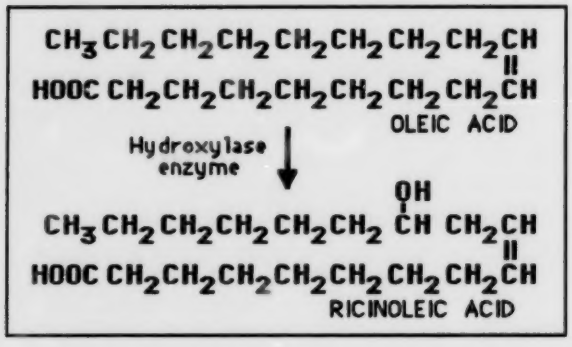


In untransformed (normal) Brassica *only 2 erucic acid units can be inserted into storage triglycerides, limiting the erucic acid content. By inserting the gene for the requisite enzyme, a transformed* Brassica *with 3 erucic acid units becomes possible.*

5.3.4 Ricinoleic Acid (E. W. Underhill)

Ricinoleic Acid (12-hydroxyoleic acid) is an industrially important substance derived uniquely from castor plant (*Ricinus communis*). This is a tropical species, so all ricinoleic acid used in temperate countries is imported. The biosynthesis of ricinoleic acid in plants involves a single enzyme step - and therefore a single gene step - from the common oleic acid. The hydroxy-acid is therefore an inviting target for genetic engineering into temperate crops such as Brassica.

In collaboration with the Plant Research Laboratory (Michigan State University) we demonstrated conversion of oleic acid via oleyl-coenzyme A to ricinoleic acid using cell-free preparations of castor plant. The hydroxylase enzyme was proved to be a mixed function oxidase through mass spectrometric analysis of the product formed from 18-deutero-oleyl coenzyme A in the presence of oxygen-18.



Formation of ricinoleic acid from oleic acid in plants.

5.3.5 Bioassembly of Storage Lipids (D. C. Taylor)

Microspore-derived embryos of *B. napus* cv. Reston, showed fatty acid and TAG accumulation profiles very similar to those observed in embryos during seed development. However, in contrast to zygotic embryo systems, the microspore embryo system efficiently incorporated radiolabeled erucic acid into TAGs *in vitro*. Activity has been localized in both microsomal and oil body fractions of microspore-derived embryos. Perhaps of greatest significance has been our finding that the DGAT efficiently converted 1,2-dierucin to trierucin in a homogenate of microspore-derived embryos. Thus,*B. napus* engineered to incorporate erucic acid into the sn-2 position will be capable of producing trierucin.

We surveyed three species (high erucic acid rapeseed, nasturtium and meadowfoam) for the ability to incorporate erucyl varieties into the middle position on the glycerol backbone, and confirmed that rapeseed was deficient in this capacity. In contrast, meadowfoam was able to carry out the reaction due to an erucyl CoA-LPAT activity 10-fold greater than that in *B. napus*. Significantly, the 18:1-CoA-LPAT activity was identical in both oilseeds. Such differences in LPAT acyl specificity could account for the high proportion of 22:1 at the middle position in meadowfoam triacylglycerols.

In our mutant work, *A. thaliana* seeds were mutagenized and grown to maturity. From the new seeds we obtained more than 10,000 third-generation seeds for screening.

Gas chromatographic analysis detected 35 mutants with altered seed fatty acid composition. Most mutants exhibited one of three distinct phenotypes: deficiencies in oleate elongation, oleate desaturation, or in linoleate desaturation. In addition we obtained an unusual mutant with levels of linolenate elevated at the expense of oleate and the corresponding C20 fatty acid.

In 35 putative mutants the fatty acid composition of the fourth generation seeds was indistinguishable from that of previous-generation seeds, indicating a stably inherited trait. Complementation analyses indicated that the majority of the mutants had a lesion at the same locus.

5.3.6 Elimination of Glucosinolates

(E. W. Underhill, J. W. D. GrootWassink)

The goal of this research has been development of zero glucosinolate plants. The specific objectives for 1989/90 included purification of two pathway enzymes, a glucosyltransferase and a sulfotransferase, production of specific monoclonal and/or polyclonal antibodies as probes for gene isolation, and isolation of seed lines devoid of glucosinolates.

Brassica campestris cv R-500 seed was mutagenized with ethyl methanesulfonate, and field propagated, then 3-week-old second-generation seedlings were screened for a metabolic block in the second half of the glucosinolate pathway. The assay was based on the conversion of administered 2-nitrobenzaldoxime to its corresponding glucosinolate. Unexpectedly, no blocked mutants were found.

A. thaliana mutants were similarly screened, and some were found to have very low to 'zero' glucosinolate levels. Seeds of lines having reduced levels have now been planted for analyses to determine levels of glucosinolates in progeny plants.

Purifying the glucosyltransferase and sulfotransferase enzymes proved to be a major challenge because contaminating protein constituted the bulk of 'purified' samples, and recoveries were very low. Accordingly, polyclonal production was dropped in favour of monoclonals. Monoclonal antibodies have been produced that bind the enzymes but these have not been proved to inhibit their activities.

5.4 Plant Cell Metabolism Project

5.4.1 Personnel:

Research Officers: *W.G.W. Kurz, J. Balsevich, V. DeLuca, R.Tyler, C. Nessler (Visiting)*
Research Associates: *S.Chevadej, N. Paiva, I. Roewer*
Technical Officers: *G. Bishop, B. Chatson, D. Dymond, J. Magus, B. Panchuk*
Summer Assistants: *N. Cloutier, K. Carrusca*
Guest Researchers: *G.Berry, N.Cheng, M. Dethier, P. Englund, D. Epp, L. Friesen, K. Giles, D. Novakovski, J. Park, D. Songstad, S. Tallevi.*

5.4.2 Introduction

The objectives of this project have been to investigate metabolic pathways in plant cells and to develop methods for their control and use in the commercial production of phytochemicals by cell culture processes.

The project entered its final year in 1989/90, having successfully achieved all its scientific objectives and having generated and transferred a large amount of new industrial technology. During the year our group conducted transformation studies (using our previously-developed gene for tryptophan decarboxylase) to determine the feasibility of engineering metabolite pool sizes in plants. However, most efforts during the year were devoted to completing technology partnership activities with Vipont Canada Ltd., BioMega Inc., and Intrinsic Research and Development Ltd.

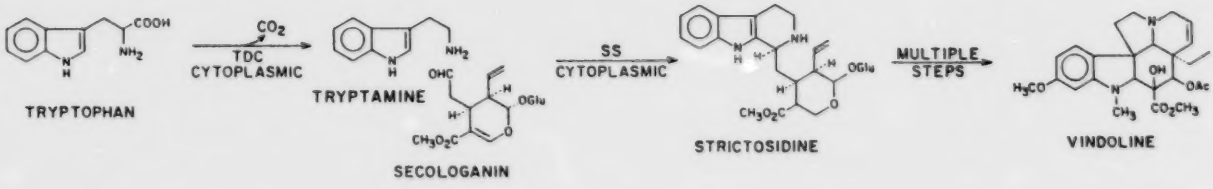
5.4.3 Tryptophan Decarboxylase expression in transformed tobacco (V. Deluca, C. Nessler)

We wished to determine whether control of metabolic "junction" pathway choices near tryptophan could alter alkaloid content or indoleacetic acid levels in plants. A full-length cDNA clone encoding tryptophan decarboxylase (TDC; EC.1.1.28) from *Catharanthus roseus* ,driven by the cauliflower mosaic virus 35S promoter, was introduced into tobacco. Young, fully expanded leaves of transformed plants had from 4 to 45 times more TDC activity than controls. Tryptamine content was directly proportional to TDC specific activity in these plants, with the highest level of tryptamine observed more than 200 times that of controls. Growth and development of cauliflower mosaic virus 35S-TDC transgenic plants remained normal regardless of their increased tryptamine content, and no significant differences in indoleacetic acid levels were detected between high tryptamine and control plants. Thus an increase in tryptamine pool size does not appear to affect auxin levels in tobacco.

5.4.4 Evaluation of surface immobilization of plant cells (W.G.W. Kurz, R. T. Tyler)

Studies on immobilized plant cell bioreactors, carried out in collaboration with the Biotechnology Research Institute in Montreal, concluded in 1989/90. During the two-year program, both periwinkle *(Catharanthus roseus)* and poppy (*Papaver somniferum)* cells were successfully cultured in 2-litre bioreactors. Overgrowth of the immobilization matrix was a chronic problem in both systems, and efforts to find culture conditions to circumvent this problem were not entirely successful. For periwinkle, use of production media proved more effective than fungal elicitation in inducing synthesis of indole alkaloids; elicitation was required to induce sanguinarine production in poppy cell cultures. In neither case did immobilization consistently improve metabolite production over that seen in similarly treated suspension cultures.

Secondary products synthesized by immobilized cultures were substantially retained within the cell vacuole so that little release of metabolites into the



culture medium occured as long as cultures remained viable. A continuous product recovery system using a hydrophobic resin was developed and applied to immobilized poppy cultures, giving much improved sanguinarine release along with enhanced and extended culture viability.

In collaboration with G. P. Slater we tested synthetic elicitors made by incomplete hydrolysis of chitin, a major constituent of *Botrytis* and other fungi known to yield elicitors upon hydrolysis. Since many fungal contain chitin, it was proposed that fungal hydrolysates might contain oligomers of N-acetylglucosamine which contributed to elicitation. Stimulation of sanguinarine production by crude hydrolysates of chitin confirmed that it is a readily accessible source of elicitor. Dr. Slater's laboratory prepared the candidate materials for tests.

Hydrolysis of chitin in concentrated hydrochloric acid yielded three times as much product as hydrolysis in 2N trifluorcacetic acid. Inclusion of tetramethyl urea increased the proportion two- or six-unit oligomers but the overall yield of hydrolysate decreased by 50%. A portion of the hydrolysate was re-acetylated to replace any N-acetyl groups lost during hydrolysis, but tests subsequently indicated that elicitation activity was greater in the non-reacetylated product. Hydrolysate was separated on gel to give the various oligomers up to seven units. We found that only the high members caused significant elicitation.

5.4.5 Alkaloid biosynthesis (J. Balsevich)

Our focus of interest remained the catharanthine-vinblastine anti-cancer alkaloids because of their use in cancer therapies. We transferred technology for production of these to a Canadian company through a licensing agreement where expertise in growing, harvesting, and processing *Catharanthus* plants for production of commercial quantities of indole alkaloids was passed on to the company. As well, we acted as project managers for trial runs involving 33 000 plants. Upon harvest the plant material was extracted and processed to provide kilogram amounts of indole alkaloid salts. Separation of alkaloids was performed in our laboratories by company personnel.

Screening of *Catharanthus* plants led to the discovery of a new alkaloid found to be a vindoline dimer linked through a unit of secologanin. However, it seemed possible that it might be an artifact arising from condensation of free secolaganin with vindoline.

5.4.6 Extramural interactions

During the year we were involved in three industrial contracts (Vipont Research Laboratories; BioMega Inc; Intrinsic Research and Development Ltd.) with a total value of more than $500,000 including $130 000 spent in our premises. One team member (DeLuca) participated in an NSERC grant, while another (Kurz) spent six months in Japan as an invited visiting professor at Kyoto University.

Other collaborations through the year involved the University of Saskatchewan, Universite de Montreal, Concordia University, the Biotechnology Research Institute, and the University of Braunschweig, Germany (Dr. U. Eilert).

6. Transgenic Plant Centre

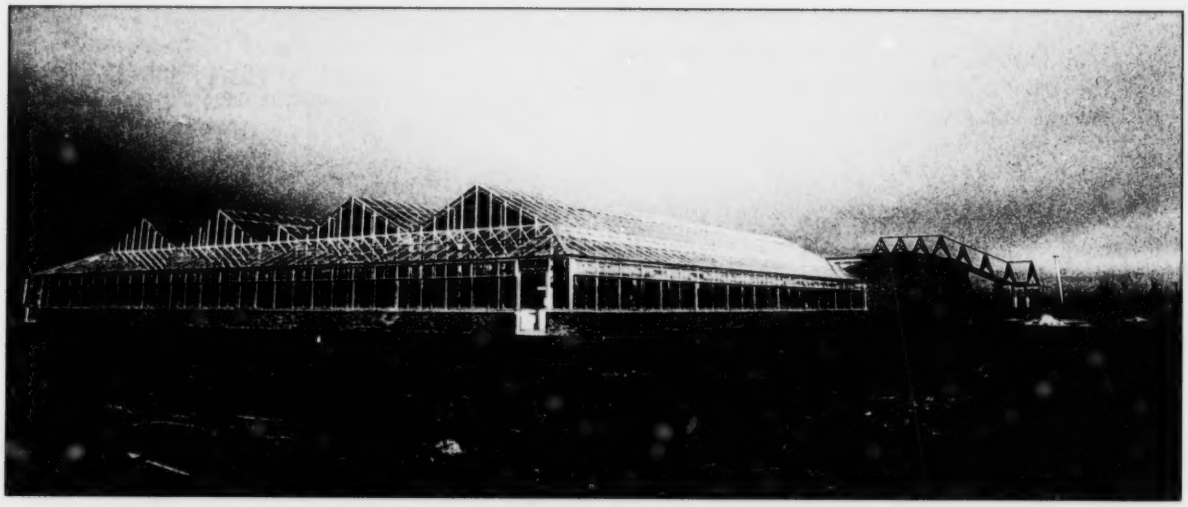
Head: Dr. W. A. Keller

6.1 Overview

As the Institute began to produce prototype new plants from its research program in 1987 the need arose for a good facility in which to propagate and assess biotechnically-produced material. In autumn 1988 NRC and the Saskatchewan Economic Development Corporation (SEDCO) first discussed these needs in the context of an agribiotechnology complex planned by SEDCO. Following joint planning, a complex was constructed which offered safe and secure research greenhouses for year-round experimentation, and adjacent laboratories and offices. SEDCO named this the Kristjanson Biotechnology Complex. Of the 2500 square metres created, PBI took a lease on 1 000 m² to establish a Transgenic Plant Centre. Equipping of the Centre was made possible by a special allocation of $0.3 million from the NRC Biotechnology Program, and the operation was opened in February 1990.

An important aspect of the Centre has been its availability for use by extramural plant biotechnology groups on a cost-recovery basis.

By year-end nearly 20 000 plants had been installed in the greenhouses and agreements for use of the Centre facilities had been concluded with four firms (Philom Bios Inc., Prairie Plant Systems Inc., MicroBio Rhizogen, Saskatchewan Wheat Pool.)



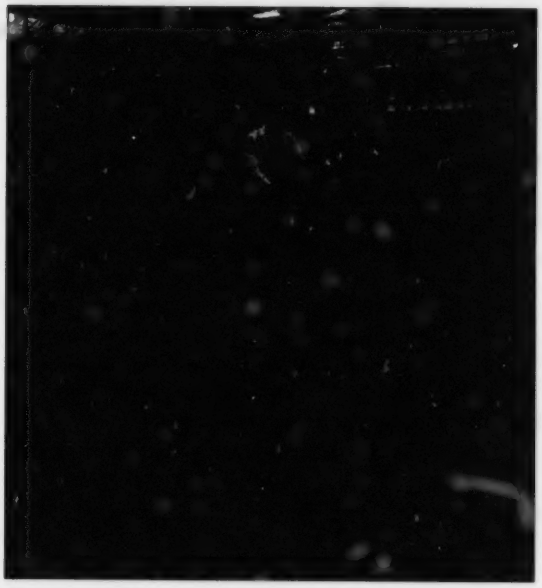
The Transgenic Plant Centre is housed in the Kristjanson Biotechnology Complex in Saskatoon.

6.2 Personnel and Facilities

As Head of this new Section NRC selected Dr. Wilfred Keller; Mr. Jerry Shyluk became the Technical Manager on-site. By March 1990 approximately 20 000 plants had been installed in our greenhouses. Four firms have signed co-occupancy agreements for space in the Centre and at year-end negotiations were in progress with two other companies. Next year's Report will give a full description of this section's activities during its initial year.

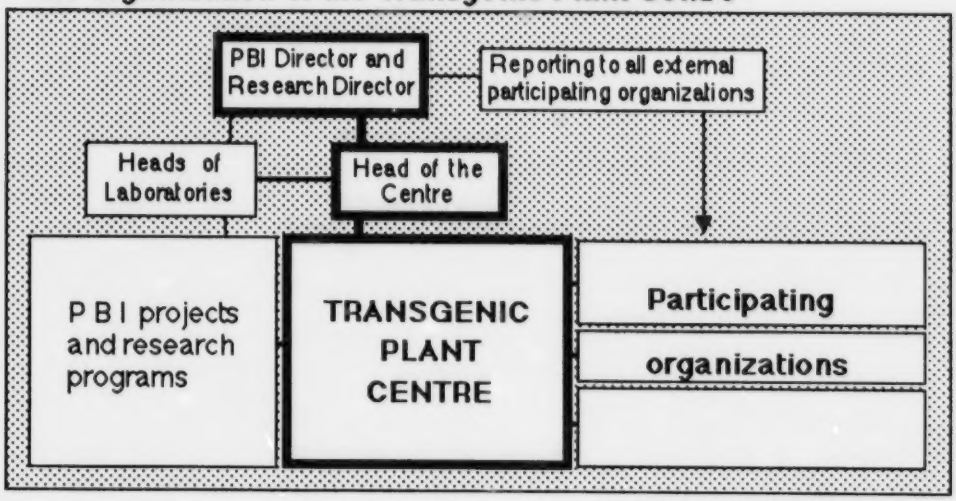
The greenhouses have capacity for about 100 000 plants and nearby growth chambers offer additional controlled-environment growth space for plants. Computer-controlled watering and temperature setting is supplemented by retractable sunshades and an automatic misting system, allowing many kinds of plants to grow well.

A project developing biotechnologies for Brassica species was established with the Centre, and had just begun at year-end. In addition a joint NRC-SEDCO steering committee was set up to foster co-operation and co-ordination of the Centre with other operations in.



Technical Officer Delwin Epp checks the watering equipment at the Transgenic Plant Centre.

The organization of the Transgenic Plant Centre



7. Business and Outreach Section

Head: Dr. W.G.W. Kurz

7.1 Overview

The Plant Biotechnology Institute engages in research business and scientific outreach as part of NRC's mandate to assist and promote research and development for Canada. Our activities in these spheres open our program and our facilities to the Canadian plant biotechnology community, and in particular to industry and university researchers. Outreach also helps us to access the best international research for adaptation to Canadian needs.

Research business dealings have become an integral part of PBI's operations. The Business and Outreach Section was established in 1988 to consolidateseveral individual program initiatives and to integrate their planning and operation. The growing volume of external investment at PBI, well above $2 million in 1989/90, indicates that our research has found strong recognition. The increasing choice of the Institute as a location for industrial research projects demonstrates our program's appeal to the biotechnology business community.

The opening of the Transgenic Plant Centre during 1989/90, and further strengthening of the Business and Outreach Section itself, should maintain or even increase this momentum in 1990/91, notwithstanding the serious financial problems which currently exist throughout the Canadian agricultural sector.

7.2 Technology Development

7.2.1 Biotechnology Development Unit

(W.G.W. Kurz, J. S. Katz)

The main aim of this unit is to maximize PBI's technology transfer opportunities. The unit promotes and co-ordinates most of PBI's activities which have a research/business linkage. These include industrial liaison, international liaison, development of agreements for joint research and for technology licensing, management of the Plant Biotechnology Fund, and conducting marketing and technology forecasting studies. Aspects of these activities are carried out in concert with the NRC Marketing Office (Ottawa, Ontario), itself a new creation in 1989, and we initiated a marketing contract with Tradex International (Calgary, Alberta) to assist our efforts in attracting international investments to Canada.

During 1989/90 there were more than eighty business sector visits to PBI of which nearly half involved the unit directly. PBI staff undertook many visits in return. Contractual agreements for research brought a half million dollars worth of investment to the Institute, spread over nearly all our projects, and agreements have already been signed guaranteeing at least this investment level in 1990/91. The Table on page 35 describes the major project collaborations supported in this way.

Protection of Crown technologies by patenting reached record levels this year. Some cases were pursued directly by NRC via firms in Montreal; others were handled by Canadian Patents and Development Limited in Ottawa. Of nine new cases active at year-end, four had proceeded to filings; two patents were obtained during the year, bringing to eighteen the

number of NRC/CPDL patents-in-force originating at PBI. The year's new protected technologies are listed at the end of Chapter 9 of this Report.

An exciting project of the unit was the publication and ensuing discussion of its national plant biotechnology study led by J. S. Katz. "Plant Biotechnology in Canada : Prospects for the 1990s" elicited high interest from all sectors and still is being discussed by the community. This report has been an important guidepost to PBI and other organizations in planning their research programs and setting their goals for the next decade. A similar study of research and technical information needs of the Canadian horticultural industry in the area of plant micropropagation, carried out by the unit during 1989/90, has now resulted in a national co-operative action plan.

7.2.2 Agreements for research and services (W.G.W. Kurz, W. F. Steck)

Licensing of some technologies occurred, and several earlier commercial exploitation licenses for elite (but non-transgenic) plants were renewed. Non-commercial use of NRC research materials developed at PBI was the topic of twenty-three materials agreements involving a wide variety of chemical standards, biochemicals, vectors and DNA sequences.

Other services provided at cost to Canadian clients under NRC's "cost recovery" policies included mass spectrometric sample analysis, nuclear magnetic resonance spectrometric analysis, custom synthesis of DNA single strands, rentals of laboratory/office space and laboratory equipment, co-occupancy access to transgenic greenhouse space, and research materials purchasing services. No off-site consultancies by staff were required in 1989/90.

The Institute and its staff provided a large volume of services without charges: training and supervision of guest researchers, teaching in specialty topics, advice and reviewing of manuscripts and proposals for funding on behalf of journals, the Natural Sciences and Engineering Research Council (NSERC), the International Development Research Council (IDRC), the Canadian Centres of Excellence Program, three federal departments, six provincial agencies, five international agencies, and twenty-three private sector companies. PBI staff served on more than fifty scientific boards, councils, commissions and advisory committees during the year, as agents of the National Research Council. The value of loans of equipment from PBI's inventory exceeded $80 000 this year, and gifts of several thousand dollars worth of research instruments to Canadian universities were made possible by new enabling regulations. In a host of small services rendered during the year, business and outreach became difficult to distinguish.

7.2.3 The Plant Biotechnology Fund (W. G. W. Kurz)

This Fund was established in 1983 to enable the Institute to contract-out parts of research projects for performance by other Canadian research groups, where this could be done better, more quickly or more economically than would be possible through in-house activities. It is not a "dedicated" fund but rather a discretionary expense taken from the overall core operational allocation made by NRC to our Institute. As the illustration below shows, the Fund began to be eroded in 1986/87 when the National Research Council entered a protracted (and continuing) period of financial constraints. By 1989/90 funds available for contracting-out had diminished drastically, and in addition the cost of in-house research had become significantly less than that of contracted research. As a result the Institute decided to terminate the Fund at the end of the fiscal year. Contracting-out on a case by case basis remains but the Fund as an instrument for systematic, competitive purchase of new intellectual property for NRC has been terminated. Two contracts were completed in 89/90 :

Abscisic acid analogs: Biological Testing
Contract value: $125 250
Principal investigator: Dr. L. V. Gusta, Crop Development Centre (University of Saskatchewan)
PBI Scientific Advisor: Dr. S. R. Abrams

Biomedical genetics of glucosinolates in *Arabidopsis thaliana* and Brassica spp.
Contract value: $56 748
Principal investigator: Dr. G. Haughn, Dept. of Biology (University of Saskatchewan)
PBI Scientific Advisor: Dr. E. W. Underhill

7.3 Outreach Activities (L. Blashill)

The Institute maintained seven outreach activities through 1989/90. These were involvements with universities via adjunct appointments; the Affiliated Scientists program; the Guest Research program; outreach publications; participation in science events; extramural research postings; and the invited seminar series.

7.3.1 Adjunct appointments

Most PBI Research Officers held adjunct professorships during the year. They were :

Dr Suzanne Abrams - *University of Saskatchewan (Chemistry)*
Dr Fred Constabel - *University of Saskatchewan (Applied Microbiology & Food Science)*
Dr William Crosby - *University of Saskatchewan (Biology)*
Dr David Dunstan - *University of Calgary (Biological Sciences)*
Dr Fawzy Georges - *University of Saskatchewan (Biochemistry)*
Dr Jan GrootWassink - *University of Saskatchewan (Applied Microbiology & Food Science)*
Dr Sean Hemmingsen - *University of Saskatchewan (Microbiology)*
Dr Shaun Hobbs -University of Saskatchewan (Crop Science & Plant Ecology)
Dr Kuo-Nan Kao - *University of Saskatchewan (Crop Science & Plant Ecology)*
Dr Kutty Kartha -*University of Saskatchewan (Horticultural Science)*
Dr Wilfred Keller - *Carleton University (Biology) and Guelph University (Crop Science), University of Saskatchewan (Crop Science & Plant Ecology)*
Dr Wolfgang Kurz - *University of Saskatchewan (Applied Microbiology & Food Science)*
Dr John Mahon - *University of Saskatchewan (Crop Science & Plant Ecology)*
Dr. Samuel MacKenzie -*University of Saskatchewan (Biochemistry)*
Dr Louise Nelson - *University of Saskatchewan (Soil Science)*
Dr Gopalan Selvaraj - *University of Saskatchewan (Crop Science & Plant Ecology)*
Dr Edward Underhill - *Simon Fraser University (Biological Sciences)*

7.3.2 Affiliates, Guests and Visiting Researchers

To complement adjunctships, PBI invites outstanding extramural Canadian scientists to become Affiliates of the Institute. Members of this group at the end of 1989/90 are listed on page 7 of this report. The 1989 annual meeting of Affiliates was held 9 August, 1989, with the topic of discussion being "Genetic Transformation".

Guest Researchers for the year are listed on page 7. This year about half of guests were sponsored by industry, the remainder being graduate students, university personnel and international guests. NRC paid no salaries or stipends to any of these guests but did provide workspace, basic experimental materials and some laboratory services.

Each year PBI invites an outstanding external scientist to spend 6 to 12 months as a salaried Visiting Research Officer at the Institute. This year's VRO was Professor Craig Nessler (Texas A & M University) who has been working in the Plant Cell Metabolism project.

7.3.3 Major PBI events during 1989/90

5 April 89 -Official visit of the Italian Biotechnology Delegation during its cross-Canada tour.
12 May 89 -Official visit of HRH Princess Chulabhorn of Thailand and her party, as part of a scientific visit to Canada.
7 June 89 -Meeting of the PBI Board of Governors.
12-15 June 89 -Participation in Global Agricultural Technology Exposition at Saskatoon, including group tours of the Institute.
9 August 89 - Second annual meeting of the PBI Affiliated Scientists at the Institute.
25 September 89 -Participation at official opening of the SEDCO Kristjanson Biotechnology Complex.
1 November 89 -Visit by Directors of the Canola Council of Canada.
15 February 90 -Official opening of the Transgenic Plant Centre.
26 March 90 -Visit by delegates to the Canada-Japan agricultural biotech workshops.

7.3.4 Seminar Series 1989/90

The Institute sponsored, in addition to short summer seminars for students and guests, a main series of seminars presenting internal and external research. Dr. Fred Constabel was the organizer. An estimated 1100 people attended the series. Information on the speakers and topics appears below.

19 April 89: **DR G. BROWN** (McGill University, Montreal): RNA plasmids in plant mitochondria.
26 April 89: **DR C.R. SOMERVILLE** (DOE Plant Research Lab, Michigan State University): Arabidopsis as a tool to study plant molecular biology and plant physiology.
20 September 89 : **DR DENNIS MURPHY** (Dep't of Biological Sciences, University of Durham, U.K.) : Regulation of the biosynthesis and modification of storage products in oilseeds.

27 September 89 : **DR FAWZY GEORGES** (NRC Plant Biotechnology Institute, Saskatoon) : Unilinkers, switchable transformation vectors.

3 October 89 : **DR VERN SELIGY** (NRC Division of Biological Sciences, Ottawa) : Genetic engi neering of glycosidases.

11 October 89 : **DR WILLIAM LANGRIDGE** (Dep't of Genetics, University of Alberta, Edmonton) : Electroporation as a method for the stable transformation of plant cells.

25 October 89: **DR JINESH JAIN** (PBI, NRC, Saskatoon): Glucosinolate biosynthesis in oilseed Brassicas

8 November 89: **DR HANS REMPEL** (PBI, NRC, Saskatoon): Induction of the virulence region of pTiC58 and its role in oncogenicity

22 November 89: **DR ATTA HUSSAIN** (PBI, NRC, Saskatoon): Chitinase and glutamate dehydro genase induction in ethylene stressed *Brassica napus*.

5 December 89: **DR PAUL YOUNG** (Dep't Biology, Queen's University, Kingston): Molecular genetic analysis of sodium transport in the yeast Schizosaccharomyces pombe.

6 December 89: **DR JANET TAYLOR** (PBI, NRC, Saskatoon): Analysis of the genetic variation between isolates of the blackleg causal organism *Leptosphaeria maculans*.

10 January 90: **DR ROBERT P ADAMS** (Baylor University, Waco, Texas): Must our tropical plant genetic resources go the way of the dinosaur? DNA Bank-Net - an alternative.

17 January 90: **DR KAY WALKER SIMMONS** (U.S.D.A. Pullman, WA): Effects of ABA on gene expression in dormant and non-dormant wheat grains

17 January 90: **DR KAY WALKER SIMMONS** (U.S.D.A., Pullman, WA): Monoclonal antibody assay for ABA: Practical aspects

2 February 90: **DR LESTER TAYLOR** (Research Triangle Park, NC): Application of mass spectrometry and MS/MS to structural analyses and metabolics and peptides

22 February 90: **DR DEREK BEWLEY** (University of Guelph): Molecular biology of desiccation

28 February 90: **DR DAVID A SOMERS** (St. Paul, Minnesota): Herbicide resistance in corn

7 March 90: **DR KENNETH GILES** (Horticulture Sciences, University of Saskatchewan, Saskatoon) : Micropropagation

14 March 90: **DR STEVE KNAPP** (Oregon State University, Corvallis) : *Cuphea viscosissima:* A new crop and model system for understanding medium-chain fatty acid synthesis

22 March 90: **DR WILLIAM RERIE** (Dept. Botany, Australian National University, Canberra) : Expression of seed storage protein genes in transgenic plants.

7.3.5 Outreach Publications

We continued producing the *PBI Bulletin* with new emphasis on its role as a marketing publication and an information vehicle for program news of interest to clients and collaborators. The *Annual Report for 1988/89* was issued in both official languages in September 1989.

Several special items published during the year included summaries of the activities of each Institute project; a prospectus of biotechnology available from PBI; and a summary of opportunities for commercial development of abscisic acid analog technology. We provided national and international news media with written as well as illustrative material for use in biosciences stories.

Mrs. Blashill's office responded to hundreds of inquiries about the Institute program and sent out information on request to firms and research groups.

8. Support Services

Administrative Manager: Mrs. Joan L. Yorke

8.1 Overview

The Support Services section provides the daily operational needs of the Institute : program administration, office services, staffing, staff training programs, building services, supply, security, transport, finance, research publication services and computer services.

As a Crown corporation, the National Research council is required to manage and administer its programs in accordance with the policies and standards of the Government of Canada under the requirements of the Privacy Act, the Access to Information Act, and many regulations. We have ongoing communications with our Ottawa headquarters. The Section provides for this, while furnishing numerous office and technical services to the research projects. The group also has responsibilities for workplace safety and for the security of the Institute.

Four units provide support services, and several Institute committees form user/ provider advisory interfaces.

8.2 Service Units

8.2.1 Administrative Services

Personnel: *Joan Yorke (Administrative Manager), Gayle Berry, Rosemarie Gallays, Ed Knapp (Research Illustration), Dawn Larson, Debbie McLeod, Kathryn Seabrook, Kathy Wilson*

We implemented NRC's new job classification system during the year, and established the Council's performance planning and review procedures throughout the Institute. Although this group remained at its 1988 size, additional duties were assumed in providing services to the Transgenic Plant Centre and in increased planning and reporting requirements of NRC. Approximately two hundred journal and conference manuscripts were produced, and five thousand reprint requests were answered.

Our group assisted in the reception and training arrangements for more than one hundred guests, students and term employees who spent time at PBI in 1989/90. Because of the increased complexity of the Institute's funding systems, we named a finance officer (K. Seabrook) during the year and undertook an intensified tracking of our budgets.

8.2.2 Building Services

Personnel: *T. Martin Mallard (Superintendent of Services), Bill Brumwell, Armand Fendelet, Rick McDonald, George Reynolds.*

Our group completed renovations to two laboratories, constructed a pair of new washrooms, extended the air conditioning system, installed five growth chambers for plant research and updated some facilities in the main building. Help was also given in furnishing and readying the Transgenic Plant Centre. A rupture of the main PBI water intake line caused a major flood in March 1990 that required extraordinary repairs and clean-up by the group.

8.2.3 Computer Services

Personnel: *T. Martin Mallard (Superintendent of Services), Harry Holfeld, Wayne Stankewich.*

This small group was separated from the Building Services group in 1989/90 because of the specialized nature of its work. During the year the unit oversaw installations and repairs of the Institute's extensive systems of mini- and micro computers. A

survey of needs for 1990 through 1995 was carried out and advice for a plan of action for this period was given attention.

8.2.4 Supply Services:

Personnel: *W.S. (Bill) Wiley (Supply Officer, to January 1990), Cheryl Bitz, Olga Coates, Irene Howe, Terry Mazurkevich, Jim McHarg (Supply Officer, from January 1990)*

Our unit saw to all tendering, ordering and delivery of PBI goods purchased, carrying out more than $3 million worth of transactions in 1989/90. We also implemented and extended the new WHMIS (Workplace Hazardous Materials Information System) and PAMS (Physical Assets Management System) requirements of the Council.

Mid-way through the year, Jim McHarg succeeded Bill Wiley as unit head, and other internal rearrangements of job duties took place.

8.3 Committees

8.3.1 User/provider advisory groups

Divisional Committee on Safety. This group is part of NRC's overall organization for workplace safety and health. It advises the Administrative Manager on potential risks, and has responsibilities in hazards procedures and in first aid training for staff. Members at year-end were: (Co-chairs) S. MacKenzie and J. Magus: (Secretary) B. Chatson, M. Mallard, T. Bethune, J. Hammerlindl, S. Hemmingsen, J. Yorke.

Computer Committtee - The group's role is to advise on future needs for computer systems; a representative from NRC's Institute for Research in Construction sits on this committee. During 1989/90 the Committee formulated an action plan for 1991-1995 for PBI. Members at year-end were: M. Mallard (chair), L. Adam, J. Balsevich, S. Hobbs, H. Holfield, M. Lapp, S. MacKenzie, C. Mallard.

Library Services Committee- assists the on-site platn biotechnology branch of the Canada Institute for Scientific and Technical Information (CISTI) in choosing book and journal orders which match current project needs in the Institute. Members at year end were: F. Chen (chair), S. Adam, C. Barber, L. Nelson, J. Newsted, G. Slater, D. Taylor.

The Plant Biotechnology Fund Committee assessed contracting-out proposals for 1989/90 and reviewed contract results from the previous year. But no new calls for proposals went out this year. This Committee will disappear in 1990 due to the closing down of the fund. Members were W.G.W. Kurz (Chair), F. Constabel, W.L. Crosby, D.I. Dunstan, K.K. Kartha, J.S. Katz (secretary), J.D. Mahon and E.W. Underhill.

8.3.2 Program Committees

Three research program committees at PBI facilitate inter-project planning, financing, contracting-out and the general coordination, evaluation and presentation of the whole research program.

The Planning and Review Committee is the Institute's principal committee where managers and project leaders meet monthly to advise the Management of PBI on some matters and to take action collegially. The group has major responsibilities in program planning and in annual assessments of project performance. Members for the year were: F. Constabel (chair), S. Abrams, D. Dunstan, S. Hemmingsen, K. Kartha, W. Keller, W. Kurz, J. Mahon, S. MacKenzie, G. Selvaraj.

The Senior Manager's Group. In recognizing the increasing role of its Section Heads in science management and to provide better channels and support for this role, PBI formed a Senior Manager's Group. The group has major responsibilities in Institute policy discussion and group decision making. Members were: W. Steck (chair), S. Abrams, F. Constabel, K. Kartha, W. Keller, J. Mahon, J. Yorke.

PBI's Special Events Committee assisted with several tours, workshops and exposition events during 1989/90. Among these events were no less than seventeen business tours, meetings of internal and external boards and all the events listed in section 7.3.3. Members at year end were L. Blashill (chair), J. Condie, L. Hoffman and L. Steinhauer.

9. Publications and Patents in 1989

Publications

During 1989, Institute staff members authored or co-authored 98 articles of which more than 90% appeared in refereed scientific journals. These articles are listed below. Publications of work performed by guests in the Institute are not listed unless an NRC staff member was a co-author of a paper.

1. **Abrams, S.R., M.J.T. Reaney, G.D. Abrams, T. Mazurek, A.C. Shaw and L.V. Gusta. 1989.** *Ratio of (S)-to (R)-abscisic acid from plant cell cultures supplied with racemic ABA.* **Phytochemistry 28: 2885-2889. NRCC No. 30267.**

2. **Abrams, S.R. 1989.** *Abscisic acid analogs:probes for basic research in plant science and practical applications in agriculture.* **Canadian Chemical News, July/August 15-17.**

3. **Archambault, J., B. Volesky and W.G.W. Kurz. 1989.** *Surface Immobilization of plant cells.* **Biotechnology & Bioengineering 33: 293-299.**

4. **Attree, S.M., D.I. Dunstan and L.C. Fowke. 1989.** *Initiation of embryogenic callus and suspension cultures, and improved embryo regeneration from protoplasts, of white spruce (Picea glauca).* **Canadian Journal of Botany 67: 1790-1795.**

5. **Attree, S.M., Dunstan, D.I. and Fowke, L.C. 1989.** *Plantlet regeneration from embryogenic protoplasts of white spruce (Picea glauca).* **Bio/Technology 7: 1060-1062.**

6. **Balsevich, J. and G. Bishop. 1989.** *Convenient preparation of iridodial by reduction of nepetalactone. Corroboration for the role of iridodial as an indole alkaloid precursor in a Catharanthus roseus cell suspension.* **Heterocycles 29: 921-924. NRCC No. 30007.**

7. **Balsevich, J. and G. Bishop. 1989.** *Distribution of catharanthine, vindoline and 3',4'-anhydrovinblastine in the aerial parts of some Catharanthus roseus plants and the significance thereof in relation to alkaloid production in cultured cells.* **Primary and Secondary Metabolism of Plant Cell Cultures, ed. W.G.W. Kurz. Springer-Verlag Berlin Heidelberg. pp. 149-153. NRCC No. 29595.**

8. **Bekkaoui, F., D.I. Dunstan. 1989.** *Permeabilization of Picea glauca protoplasts to macromolecules.* **Canadian Journal of Forest Research 19: 1316-1321. NRCC No. 30008.**

9. **Boothe, J.G., and D.W. Walden. 1989.** *Multivariate analysis of polypeptide synthesis in developing maize embryos.* **Theoretical and Applied Genetics 77: 495-500.**

10. **Browse, J., L. Kunst and C. R. Somerville. 1989.** *A mutant of Arabidopsis deficient in the chloroplast 16:1/18:1 desaturase.* **Plant Physiology 90: 522-529.**

11. **Browse, J., L. Kunst, S. Hugly and C. Somerville. 1989.** *Modifications to the two pathway scheme of lipid metabolism based on studies of Arabidopsis mutants. Proceedings of the 8th International Symposium on the Biological Role of Plant Lipids,* **In: Biological Role of Plant Lipids (P.A. Biacs, K. Gruiz, T. Kremmer, eds.) Akademial Klado, Budapest and Plenum Publishing Corp. New York and London, pp. 335-339.**

12. **Chanway, C.P., R.K. Hynes and L.M. Nelson. 1989.** *Plant growth-promoting rhizobacteria: effects on growth and nitrogen fixation of lentil (Lens esculenta Moench) and pea (Pisum sativum L.).* **Soil Biology and Biochemistry 21: 511-517. NRCC No. 29581.**

13. **Chibbar, R.N., P.L. Polowick, J. Newsted, J. Shyluk and F. Georges. 1989.** *Identification and isolation of a unique esterase from the medium of non-embryogenic cell line of cultured carrot cells.* **Plant Cell Tissue & Organ Culture 18: 47-53. NRCC No. 30002.**

14. **Chibbar, R.N., P.L. Polowick, W.J., Newsted, F. Georges, J. Shyluk and F.A. Constabel. 1989.** *"Unique esterase from the medium of a non-embryogenic cell line of culture carrot cells".* **ASPP & CSPP Joint annual meeting, July 1989, Toronto, Canada. Plant Physiology 89 (4S):9.**

15. **Cutler, A.J., M. Saleem, E. Kendall, L.V. Gusta, F. Georges and G.L. Fletcher. 1989.** *The winter flounder antifreeze protein improves the cold hardiness of plant tissues.* **Journal of Plant Physiology. Journal of Plant Physiology 135: 351 - 354. NRCC No. 28869.**

16. **Cutler, A.J., M. Saleem, M.K. Loewen and M.A. Coffey. 1989.** *Role of oxidative stress in cereal protoplast recalcitrance.* **Plant Cell Tissue and Organ Culture 18: 113-127. NRCC No. 29583.**

17. **Cutler, A.J., M. Saleem, E. Kendall, L.V. Gusta, F. Georges and G.L. Fletcher. 1989.** *Enhancing plant cold hardiness with the winter flounder antifreeze protein.* **Plant Physiology 89: 32S.**

18. **Delisle, J., J.N. McNeil, E.W. Underhill and D. Barton. 1989.** *Helianthus annuus pollen, an oviposition stimulant for the sunflower moth, Homoeosoma electellum.* **Entomologia Experimentalis et Applicata 50: 53-60. NRCC No. 28705.**

19. **DeLuca, V., N. Brisson, J. Balsevich and W.G.W. Kurz. 1989.** *Regulation of vindoline biosynthesis in Catharanthus roseus: molecular cloning of the first and last steps in biosynthesis.* **Proceedings of the II International Symposium on Primary and Secondary Metabolism of Plant Cell Cultures, W.G.W. Kurz, ed., Springer-Verlag, Berlin, Heidelberg, pp. 154-161. NRCC No. 29490.**

20. DeLuca, V. 1989. *Regulation of metabolite flow.* **Proceedings of the II International Symposium on Primary and Secondary Metabolism of Plant Cell Cultures, W.G.W. Kurz, ed., Springer-Verlag, Berlin, Heidelberg, pp. 301-302. NRCC No. 30263.**

21. DeLuca, V., C. Marineau and N. Brisson. 1989. *Molecular cloning and analysis of cDNA encoding a plant tryptophan decarboxylase: Comparison with animal dopa decarboxylases.* **Proceedings of the National Academy of Science USA 86: 2582-2586. NRCC No. 30006.**

22. Dunstan, D.I., T. Bethune, S. Kikcio, and M.S. Lapp. 1989. *In vitro shoot formation among 17-year-old Douglas-fir provenances.* **Canadian Journal of Forest Research 19: 1299-1302. NRCC No. 29587.**

23. Ellis, R.J., S.M. van der Vies and S.M. Hemmingsen. 1989. *The molecular chaperone concept.* **Biochemical Society Symposia 55: 145-153.**

24. Ellis, R.J. and S.M. Hemmingsen. 1989. *Molecular chaperones: proteins essential for the biogenesis of macromolecular structures.* **Trends in Biochemical Science 14: 339-342.**

25. Fernandez, J.A., T.G. Owen, W.G.W. Kurz and V. DeLuca. 1989. *Immunological detection and quantitation of tryptophan decarboxylase in developing Catharanthus roseus seedlings.* **Plant Physiology 91: 79-84. NRCC No. 30011.**

26. Fernandez, J.A. W.G.W. Kurz and V. De Luca. 1989. *Conformation-dependent inactivation of tryptophan decarboxylase from Catharanthus roseus.* **Biochemical and Cell Biology 67: 730 - 734. NRCC No. 30497.**

27. Flood, P.F., S.R. Abrams, G.D. Muir and J.E. Rowell. 1989. *The odour of the Muskox: A preliminary investigation.* **Journal of Chemical Ecology 15: 2207 - 2217.**

28. Gaitan, E., R.H. Lindsay, R.D. Reichert, S.H. Ingbar, R.C. Cooksey, J. Jegan, E.F. Meydrech, J. Hill and K. Kubota. 1989. *Antithyroid and goitrogenic effects of millet: role of C-glycosylflavones.* **Journal of Clinical Endocrinology and Metabolism 68: 707-714.**

29. Georges, F., R.N. Chibbar, W.J. Newsted and F. Constabel. 1989. *Novel approach to the ligation of single-stranded DNA fragments by T_4 DNA ligase - DNA mobile multiple-restriction fragments: "un-linkers" for cloning of genes.* **Nucleosides and Nucleotides 8: 1427-1440.**

30. Georges, F., R.N. Chibbar, W.J. Newsted and F. Constabel. 1989. *Conversion of single-stranded DNA fragments to double-stranded forms through ligation to unique hairpin loop sequences. Construction of uni-ended mobile cloning site fragments (unilinkers).* **Plant Physiology 89 (4S): 65.**

31. Good, A.G. and W.L. Crosby. 1989. *Induction of alcohol dehydrogenase and lactate dehydrogenase in hypoxically induced barley.* **Plant Physiology 90: 860-866. NRCC No. 29494.**

32. Good, A.G. and W.L. Crosby. 1989. *Anaerobic induction of alanine aminotransferase in barley root tissue.* **Plant Physiology 90: 1305-1309. NRCC No. 29499.**

33. Griffing, L.R., L.C. Fowke and F. Constabel. 1989. *Radioimmunoassay of morphinan alkaloids in Papaver somniferum hypocotyls, callus, and suspension cultures.* **Journal of Plant Physiology 134: 645-650.**

34. GrootWassink, J.W.D., G.L. Campbell and H.L. Classen. 1989. *Fractionation of crude pentosanase (arabinoxylanase) for improvement of rye diets for broiler chickens.* **Journal of the Science of Food and Agriculture 46: 289 - 300.**

35. Gummuluru, S., S.L.A. Hobbs and S. Jana. 1989. *Genotypic variability in physiological characters and its relationship to drought tolerance in durum wheat.* **Canadian Journal of Plant Science 69: 703-711. NRCC No. 29856.**

36. Gummuluru, S., S.L.A. Hobbs and S. Jana. 1989. *Physiological responses of drought tolerant and drought susceptible durum wheat genotypes.* **Photosynthetica 23: 479-485. NRCC No. 30712.**

37. Hobbs, S.L.A, J.A. Jackson, and J.D. Mahon. 1989. *Specificity of strain and genotype in the susceptibility of pea to Agrobacterium tumefaciens.* **Plant Cell Reports 8: 274-277. NRCC No. 30265.**

38. Huang, B. and W.A. Keller. 1989. *Microspore culture technology.* **Journal of Tissue Culture Methods 12: 171-178.**

39. Hugly, S., L. Kunst, J. Browse and C. Somerville. 1989. *Enhanced thermal tolerance of photosynthesis and altered chloroplast ultrastructure in a mutant of Arabidopsis deficient in lipid desaturation.* **Plant Physiology 90: 1134-1142.**

40. Ikeda, M., A.P. Tulloch and L.L. Hoffman. 1989. *A plant growth inhibitor from immature seeds of corn.* **Agricultural and Biological Chemistry 53: 569-570.**

41. Jain, J.C., M.R. Michayluk, J.W.D. GrootWassink and E.W. Underhill. 1989. *Distribution of enzymes catalyzing the glucosylation and sulfation steps of glucosinolate biosynthesis in Brassica juncea seedlings and cultured cells.* **Plant Science 62: 25-29. NRCC No. 29498.**

42. Jain, J.C., D.W. Reed, J.W.D. GrootWassink and E.W. Underhill. 1989. *A radioassay of enzymes catalyzing the glucosylation and sulfation steps of glucosinolate biosynthesis in Brassica napus.* **Analytical Biochemistry 178: 137-140.**

43. Kao, K.N. and M.R. Michayluk. 1989. *Fusion of plant protoplasts - techniques.* **Biotechnology in Agriculture and Forestry 8: 277-288. NRCC No. 29582.**

44. Kartha, K.K., R.N. Chibbar, F. Georges, N. Leung, K. Caswell, E. Kendall and J.A. Qureshi. 1989. *Transient expression of chloramphenicol acetyl transferase (CAT) gene in barley cell cultures and immature embryos.* **Plant Cell Reports 8: 429-432. NRCC No. 30706.**

45. Katovich, S.A., P.D. Swedenborg, M. Giblin and E.W. Underhill. 1989. *Evidence for E,Z-8, 10-dodecadienyl acetate as the major component of the sex pheromone of the eastern pine seedworm, Cydia toreuta (Lepidoptera: Tortricidae).* **Journal of Chemical Ecology 15: 581-590. NRCC No. 27934.**

46. Katz, J.S. and W. Steck. 1989. *Canadian survey highlights plant biotechnology requirements for the 1990s.* **Trends in Biotechnology 7: 135-137.**

47. Kendall, E.J. and B.D. McKersie. 1989. *Free radical and freezing injury to cell membranes of winter wheat.* **Physiologia Plantarum 76: 89-94.**

48. Kunst, L., J. Browse and C. Somerville. 1989. *Altered chloroplast structure and function in a mutant of Arabidopsis deficient in plastid glycerol-3-phosphate acyltransferase activity.* **Plant Physiology 90: 846-853.**

49. Kunst, L., J. Browse and C. R. Somerville. 1989. *A mutant of Arabidopsis deficient in desaturation of palmitic acid in leaf lipids.* **Plant Physiology 90: 943-947.**

50. Kunst, L., J. Browse and C. Somerville. 1989. *Enhanced thermal tolerance in a mutant of Arabidopsis deficient in palmitic acid unsaturation.* **Plant Physiology 91: 401-408.**

51. Kunst, L., J.E. Klenz, J. Martinez-Zapater and G.W. Haughn. 1989. *AP2 gene determines the identity of perianth organs in flowers of Arabidopsis thaliana.* **The Plant Cell 1: 1195-1208.**

52. Kunst, L., J. Klenz, J. Martinez-Zapater and G.W. Haughn. 1989. *Floral development in Arabidopsis thaliana: The role of the AP2 locus.* **Plant Physiology 89:(4S) 98.**

53. Kurz, W.G.W. 1989. *Bottlenecks towards commercialization of product synthesis by plant cell cultures.* **In: Primary and Secondary Metabolism of Plant Cell cultures II, W.G.W. Kurz, ed., Springer-Verlag, Berlin, Heidelberg, pp. 303-304.**

54. Le Gal, M.F. and S.L.A. Hobbs. 1989. *Cytological studies of the infection process in nodulating and nonnodulating pea genotypes.* **Canadian Journal of Botany 67: 2435-2443. NRCC No. 30304.**

55. Le Gal, M.F., S.L.A. Hobbs and C.M.O. Delong. 1989. *Gene expression during the infection process in nodulating and non-nodulating pea genotypes.* **Canadian Journal of Botany 67: 2535-2538.**

56. McAfee, B.J. and J.A. Fortin. 1989. *Ectomycorrhizal colonization on black spruce and jack pine seedlings outplanted in reforestation sites.* **Plant and Soil 116: 9-17.**

57. McGavin, M., C. Forsberg, W.L. Crosby, A.W. Bell, D. Dignard, and D.Y. Thomas. 1989. *Structure of the cel3 gene and identification of the encoded gene product endoglucanase 3 (EG3) in Fibrobacter succinogenes.* **Journal of Bacteriology 171: 5587-5595**

58. McHughen, A., M. Jordan and G. Feist. 1989. *A preculture period prior to Agrobacterium inoculation increases production of transgenic plants.* **Journal of Plant Physiology 135: 245-248.**

59. McHughen, A. and M.C. Jordan. 1989. *Recovery of transgenic plants from "escape" shoots.* **Plant Cell Reports 7: 611-614.**

60. Miki, B., B. Huang, S. Bird, R. Kemble, D. Simmonds and W.A. Keller. 1989. *A procedure for the microinjection of plant cells and protoplasts.* **Journal of Tissue Culture Methods 12: 139-144.**

61. Nehra, N.S., C. Stushnoff and K.K. Kartha. 1989. *Direct shoot regeneration from strawberry leaf discs.* **Journal of the American Society for Horticultural Sciences 114: 1014-1018.**

62. Pechan, P.M. and W.A. Keller. 1989. *Induction of microspore embryogenesis in Brassica napus L. by gamma irradiation and ethanol stress.* **In Vitro Cellular & Developmental Biology 25: 1073-1074.**

63. Pedras, M.S.C., S.R. Abrams, G. Seguin-Swartz, J.W. Quail and Z. Jia. 1989. *Phomarazine, a novel toxin from the phytopathogenic fungus Phoma lingam.* **Journal of the American Chemistry Society 111: 1904-1905. NRCC No. 29584.**

64. Pilate G., B. Sotta, R. Maldiney, M. Jacques, L. Scsaountzov and E. Miginiac. 1989. *Abscisic acid, indole-3-acetic acid and cytokinin changes in buds of Pseudotsuga menziesii during bud quiescence release.* **Physiologia Plantarum 76: 100-106.**

65. Pilate G., L. Sossountzov and E. Miginiac. 1989. *Hormone levels and apical dominance in the aquatic fern Marsilea drummondii A. Br.* **Plant Physiology 90: 907-912.**

66. Priesner, E., D.W. Reed, E.W. Underhill and H. Bogenschutz. 1989. *(Z,Z)-7,9-dodecadienyl acetate, sex pheromone of Epinotia tedella Clerck (Lepidoptera: Tortricidae).* **Journal of Chemical Ecology 15: 2457 -2464. No NRCC No.**

67. Qureshi, J.A., K.K. Kartha, S.R. Abrams and L. Steinhauer. 1989. *Modulation of somatic embryogenesis in early and late-stage embryos of wheat (Triticum aestivum) under the influence of abscisic acid and its analogs.* **Plant Cell Tissue and Organ Culture 18: 55-69. NRCC No. 30010.**

68. Qureshi, J. and S.R. Abrams. 1989. *Abscisic acid analogs: New tools for tissue culture of cereals.* **PBI Bulletin (July) 4-6.**

69. Reaney, M.J.T., L.V. Gusta and S.R. Abrams. 1989. *The effects of abscisic acid, kinetic and gibberellin on freezing tolerance of in smooth bromegrass (Bromus inermis Leyss.) cell suspension cultures.* **Canadian Journal of Botany 67: 3640 - 3646.**

70. Reed, D.W., K.A. Pivnick and E.W. Underhill. 1989. *Identification of chemical oviposition stimulants for the diamond-back moth, Plutella xylostella, present in three species of Brassicaceae.* **Entomologia Experimentalis et Applicata 53: 277-286.**

71. Roewer, I.A., W.G.W. Kurz, K. Giles and V. De Luca. 1989. *Activity of L-tyrosine and L-dopa decarboxylase in cell suspension cultures of Papaver somniferum after elicitor treatment.* **Planta Medica 55: 594.**

72. Saleem, M., A. I. Cutler, M. Coffey and M. Loewen. 1989. *Effects of DNA methylation inhibitor on cultured corn leaf protoplasts.* **Plant Physiology 89: 191S.**

73. Seitz, H.U., U. Ellert, V. De Luca and W.G.W. Kurz. 1989. *Elicitor-mediated induction of phenylalanine-ammonia lyase and tryptophan decarboxylase: accumulation of phenols and indole alkaloids in cell suspension culture of Catharanthus roseus.* **Plant Cell Tissue and Organ Culture 18: 71-78. NRCC No. 30004.**

74. Shargool, P.D. and J.C. Jain. 1989. *Purification and immunological properties of an NAD(H) dependent glutamate dehydrogenase from soybean cells (Glycine max L.).* **Plant Science 60: 173-179.**

75. Steck, W. and W.G.W. Kurz. 1989. *Plant biotechnology in the 1990's: consensus and conflict.* **PBI Bulletin, (April) 5-8.**

76. Tautorus, T.E., F. Bekkaoui, M. Pilon, R.S.S. Datla, W.L. Crosby, L.C. Fowke and D.I. Dunstan. 1989. *Factors affecting transient gene expression in electroporated black spruce (Picea mariana) and jack pine (Picea banksiana) protoplasts.* **Theoretical and Applied Genetics 78: 531-536. NRCC No. 30491.**

77. Taylor, D.C., E.W. Underhill, N. Weber, M.K. Pomeroy and L. Edwards. 1989. *Storage lipid biosynthesis in microspore-derived Brassica napus embryos.* **Plant Physiology 89: 77S.**

78. Thomas, C.M., C.A. Smith, V. Shingler, M.A. Cross, A.A.K. Hussain and M. Pinkney. 1989. *Regulation of replication and maintenance functions of broad host-range plasmid RK2.* **Basic Life Sciences 30: 261 -276. No NRCC No.**

79. Thorpe, T.A., K. Bagh, A.J. Cutler, D.I. Dunstan, D.D. McIntyre, and H.J. Vogel. 1989. *A ^{14}N and ^{15}N study of nitrogen metabolism in shoot-forming cultures of white spruce.* **Plant Physiology 91: 193-202.**

80. Toivonen, L., J. Balsevich and W.G.W. Kurz. 1989. *Indole alkaloid production by hairy root cultures of Catharanthus roseus.* **Plant Cell Tissue and Organ Culture 18: 79 - 94. NRCC No. 30009.**

81. Toivonen, P.M.A. and K.K. Kartha. 1989. *Cryopreservation of cotyledons of nongerminated white spruce (Picea glauca (Moench) Voss) embryos and subsequent plant regeneration.* **Journal Plant of Physiology 134: 766-768. NRCC No. 30259.**

82. Tsang, E.W., H. David, A. David and D.I. Dunstan. 1989. *Toxicity of antibiotics on zygotic embryos of white spruce (Picea glauca) cultured in vitro.* **Plant Cell Reports 8: 214-216. NRCC No. 30626.**

83. Tyler, R.T., U. Eilert, C.O.M. Rijnders, I.A. Roewer, C.K. McNabb and W.G.W. Kurz. 1989. *Studies on benzophenanthridine alkaloid production in elicited cell cultures of Papaver somniferum* **L. Proceedings of the II International Symposium on Primary and Secondary Metabolism of Plant Cell Cultures, Saskatoon, Saskatchewan, July 4-8, 1988, W.G.W. Kurz, ed., Springer-Verlag, Berlin Heidelberg, pp. 200-207. NRCC No. 29147.**

84. Vanzegeren, H.L., A.J. Cutler and M. Saleem. 1989. *Use of osmotically permeabilized plant protoplasts to study DNA synthesis.* **Phytochemistry 28: 323-327. NRCC No. 28384.**

85. Vézina, L.P., H.A. Margolis and B.J. McAfee. 1989. *Changes in the activity of enzymes involved with primary nitrogen metabolism due to ectomycorrhizalsymbiosis on jack pine seedlings.* **Physiologia Plantarum 75: 55-62.**

86. von Rudloff, E. and M. Lapp. 1989. *Some observations on the leaf oil terpene composition of mountain hemlock Tsuga mertensiana.* **Canadian Journal of Forest Research 19: 848-852. NRCC No. 30250.**

87. Wang, H., A.J. Cutler, M. Saleem and L.C. Fowke. 1989. *Immunocytochemical detection of DNA synthesis in plant cells.* **Journal of Plant Physiology 135: 15-20.**

88. Wang, H., A.J. Cutler and L.C. Fowke. 1989. *High frequencies of preprophase bands in soybean protoplast cultures.* **Journal of Cell Science 89: 575-580**

89. Wang, H., A.J. Cutler, M. Saleem and L.C. Fowke. 1989. *Microtubules in maize leaf protoplasts in relation to donor tissue and in vitro culture.* **Protoplasma 150: 48-53. NRCC No. 28873.**

90. Wang, H., A.J. Cutler, M. Saleem, and L.C. Fowke. 1989. *DNA replication in maize leaf protoplasts.* **Plant Cell Tissue and Organ Culture 18: 33-46. NRCC No. 28872.**

91. Wang, H., A.J. Cutler and L.C. Fowke. 1989. *Preprophase bands in cultured multinucleate soybean protoplasts.* **Protoplasma 150: 110-116.**

92. Wang, H., A.J. Cutler, M. Saleem and L.C. Fowke. 1989. *Microtubules in maize protoplasts derived from cell suspension cultures: Effect of calcium and magnesium ions.* **European Journal of Cell Biology 49: 80-86.**

93. Wang, H., A.J. Cutler, M. Saleem and L.C. Fowke. 1989. *Treatment of soybean cells with cell wall-degrading enzymes inhibits nuclear division but not DNA synthesis.* **Journal Plant Physiology 135: 404-408. NRCC No. 30492.**

94. Wang, H., A.J. Cutler, M. Saleem and L.C. Fowke. 1989. *DNA synthesis in maize leaf protoplasts.* **In Vitro 25: 32A.**

95. Waygood, E.B., S. Sharma, P. Bhanot, O.A.L. El-Kabbani, L.T.J. Delbaere, F. Georges, M.G. Wittekind, and R.E. Klevit. 1989. *The structure of HPr and site-directed mutagenesis.* **FEMS Microbiology Reviews 63: 43-52.**

96. Weber, N., D.C. Taylor, E.W. Underhill and M.K. Pomeroy. 1989. *Microspore embryos of B. napus: A model for studying triacylglycerol biosynthesis in seeds.* **Biological Chemistry Hoppe-Seyler 370: 799.**

97. Weselake, R.J., L.W. Thomson, D. Tenaschuk and S.L. MacKenzie. 1989. *Properties of solubilized microsomal lipase from germinating Brassica napus.* **Plant Physiology 91: 1303-1307.**

98. Wiersma, P.A., M.G. Schmiemann, K.L. Hurlburt, J.L. Condie, D.S.S. Raju, W.L. Crosby and M.M. Moloney. 1989. *Isolation, characterization and phylogenetic inheritance of an acetolactate synthase gene from Brassica napus.* **Molecular and General Genetics 219: 413-420.**

Patents

1990

1. Constabel, F., W.G.W. Kurz & U. F. K. Eilert. Repeated elicitor treatment, a method for semicontinuous metabolite production by plant cells cultured *in vitro*. Canadian Patent No. 496 984.

A list of all active crown patents based on PBI research is available on request from the Institute.

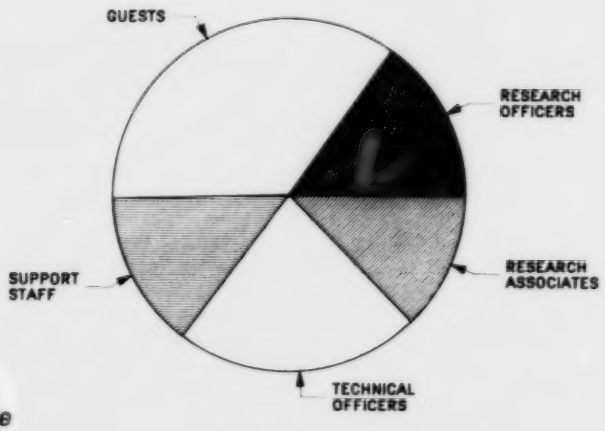
10. Canada's Investment at PBI 1989/90

The National Research Council was by far the major funding source for PBI during the year, allocating $10.5 million for upkeep of the premises, performance of research and support of NRC initiatives operating out of our location. But extramural investments at the Institute continued to grow in 1989/90 to a total of $2.8 million.

Revenues, the cash receipts received for services provided to extramural users, amounted to $126 thousand (up from $62 thousand in 88/89), while the value of research performed under contract through clients' resources rose to $509 thousand from last year's $390 thousand. Support of 52 person-years of guest research by our guests' organizations included an estimated $2.6 million in salary and benefits outlays during the year, an increase of 20% over the previous year, along with $0.2 million of associated costs.

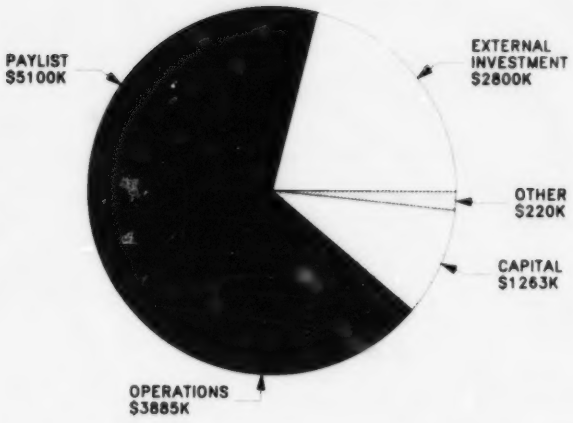
Because of NRC's difficult current financial situation, the core operational budget of the Institute was held at the level of 1988 this year, resulting in a considerable net loss of purchasing power. However, supplementary NRC "one-time" investments raised the total operational allocation to $3.9 million. NRC's expenditures for research equipment amounted to $1.3 million and paylist costs added a further $5.3 to this, completing the total of $10.5 million.

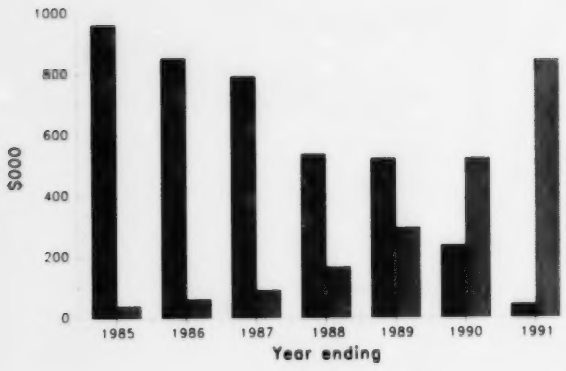
The diagrams below and on the next page show the kinds of amounts of resources both human and financial - invested at PBI in 1989/90, along with the purposes for which expenditures were made.



Human resources in 1989/90: Of the 160 person-years of labour invested, guests provided one-third and NRC staff provided two-thirds. Approximately equal numbers of industry and university guests were present at PBI during the year.

Financial expenditures in 1989/90: NRC invested $10.5 million and other organizations invested $2.8 million in salaries and operational support during the year. NRC's "operations" expenses of $3885K were accounted as follows:
Travel 225K
Info and Communications 114
Special Services Contracts 471
Rentals 278
Utilities 674
Materials and supplies 1313
Repairs and maintenance 545
R and D Contracts
Other





Contracting-in and contracting-out: Trends in the contracting of research have been strongly in favour of the former over the past six years, and we anticipate a continuation of this trend in 1990/91. At least two factors are at work: increased costs of contracted-out work relative to our in-house costs; and shortfalls in internal budgets of PBI since 1986. Since 1988 the Institute has not been able to continue the role of national "distributor" of plant biotechnology research investment.

The Year Ahead

The Plant Biotechnology Institute's business plan for the years 1990 to 1995 aims to maintain most of the research efforts described in this report, and emphasizes larger partnerships with other Canadian groups. Outputs for industrial use will continue to be our primary aim. Activities in regulation of plant genes have been assigned a high priority. In this connection a full-fledged project on Abscisic Acids is being set up.

We hope during 1990/91 to undertake our first field assessments of genetically engineered oilseed plants, to succeed in transformations of some horticultural species, and to begin to increase our efforts in regulation and control of plant gene action. On a continuing strong foundation of basic research we hope to build many industrial collaborations along with at least one or two industry partnerships of international dimensions.

Recent biotechnical advances now make a concerted effort possible on genetic engineering of barley and wheat. PBI will have a substantial project on cereal grains biotechnology beginning in 1990.

Finally, we are working in 1990/91 to prepare more space and facilities on our premises for use by the ever-increasing numbers of guest researchers and visiting research teams wishing to come to the Institute. Even though our own staff numbers are expected to drop from 110 to 106 during the year ahead, the national level of activity in plant biotechnology research will certainly continue to rise. We hope to remain a principal player in this national activity.

Printed by Houghton Boston Printers, Saskatoon, Sask.

Single copies of this report are available without charge from NRC Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Canada S7N OW9. Our facsimile number is (306) 975-4839.

Cover photo: Microspore-derived embryos of *Brassica napus*. Photo by PBI/NRC technical officer Morris Michayluk.

Cette publication est aussi disponible en francais.